PRACTICAL URANALYSES

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Fig. 1.—Apparatus used in uranalysis.

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PREFATORY NOTE

The uranalysis should be regarded as a necessary part of every diagnostic examination. Until this is done, the sick man will be denied a large part of that which scientific medicine offers him. A period of renascence passes rapidly, and we see at hand a realization of the prophecy of Osler, "It will be done more and more when we send out our students familiar by long practice with the use of the microscope and other instruments of precision."

Where antilogia, or a combination of contradictory symptoms rendering diagnosis uncertain, has existed, we have wished to call in everything which medical chemistry, microscopy, and bacteriology have given us; and it may be ventured that until this situation has confronted the average practitioner, has he been willing to look to the laboratory for aid. Inasmuch as uracrasia, or a disordered state of the urine, is commonly met in disease, it is probable that the uranalysis is more frequently attempted than any other one piece of laboratory work.

The most valuable uranalyses are not those for casts, blood and pus, but for the minor products of erroneous metabolism and excretion—proofs of disturbed function and its nature in certain tissues, or-

gans and systems, while remedial measures may yet avail. This manual is not to be regarded as anything aiming at completeness, but as a guide for the student and practitioner, in those diagnostic matters likely to be encountered from day to day. Much emphasis has been placed upon findings often considered minor, and but little attention given to questions which might interest the specialist or research worker. The reader is, however, urged to study the larger books, if he would quickly gain the laboratory spirit.

I have prepared these outlines, hoping to emphasize many of the little points which are often lost in the complexity of the large book.

B. G. R. Williams.

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PRACTICAL URANALYSES

CHAPTER I.

PROPERTIES OF THE NORMAL URINE.

What the housewife knows today concerning the use of water in the removal of filth, Dame Nature learned many centuries ago in regard to animal and plant life. In the lower forms all the products of katabolism must be soluble to be eliminated, whether through the almost invisible pellicula of the ameba or the condensed cell wall of the bacillus. In the higher forms of life the removal of waste products of metabolism has been provided for by the kidney, intestine, sweat gland, and lung. In health, the highly differentiated cells of these structures select with great care what substances they wish to excrete. That is to say while water is an important constituent of every excretion, urea is eliminated mainly by the kidney but bilirubin and indican never by the kidney. We find conditions much different in disease. Here the waste substance cannot be excreted perfectly by the proper cells, and in consequence the duty falls upon others scarcely fitted for the work. This chapter has to deal with those substances which in perfect health are eliminated by way of the kidney.

Normal Constituents of the Urine.—Normal urine must be regarded as an aqueous solution not only of certain products of katabolism but of substances which result from excess of foodstuffs. That is to say the solid constituents of the urine may be increased by overeating just as the water may be raised by drinking in excess, and the practitioner often loses sight of the fact that variations in the total solids are not always to be explained by disease. The following table will give approximately the percentage composition of normal urine (urine of a person in health and upon a proper diet). As shall repeatedly be pointed out in this book, figures are based upon examination of twenty-four hour samples:

Water	95%
Solids	5%
Urea	2.5%
Other organic constituents	.5%
Inorganic salts	2. %

Next to water, urea must be regarded as the most important constituent of the urine. It represents about eighty-five per cent of the total nitrogen eliminated by the kidneys, which means over three-quarters of the proteid waste of the body. Urea is elaborated in the liver by a combination of carbon dioxid and ammonia, two exceedingly poisonous, katabolic products. Urea is scarcely poisonous, however, but is an active diuretic. It is quite soluble in water, and occurs in the urine in perfect solu-

tion. For the clinical significance of urea variations and for calculations, see page 93.

Other organic constituents of the urine are creatinin, uric acid, hippuric acid, and certain pigments. Uric acid occurs chiefly in the form of urates of sodium and potassium.

The inorganic, or mineral constituents of the urine exist for the most part in the form of sodium and potassium salts. Thus we find such combinations with the chlorids, sulphates, phosphates, and so on. Under certain conditions, as variations in reaction or changes in diet, we may also find oxalates and carbonates. Likewise the sodium and potassium may be replaced in part in these combinations by calcium, magnesium, and ammonia, in which case the salts become less soluble or may even be precipitated before the urine is voided. For the actual amounts of the various constituents excreted each twenty-four hours, the reader is referred to page 88.

Variations Due to Ammoniacal Fermentation.—A standing urine undergoes fermentation, usually of an ammoniacal character, though there may be some variations in the actual process, depending upon the type of bacteria causing the change, temperature and other influences not so well understood. There are other types of fermentation, but this being the usual one, it is well to study it somewhat thoroughly that findings may be easily interpreted. Ammoniacal fermentation is met with most commonly in voided, standing samples of urine. In

case there is retention of urine in the bladder, the same condition may occur.

We have noted that urea, a complex and scarcely poisonous body, is elaborated in the liver from carbon dioxid and ammonia, two very poisonous products of katabolism. But it can in turn be broken down by unicellular forms of life into a number of simple products among which ammonia and carbon dioxid are chief. This is accomplished by a number of bacteria which elude classification; though it is probable that certain micrococci are most active in the process.

At any rate carbon dioxid and ammonia are set free. The former may escape in part as gas, but usually unites in considerable amounts with such calcium as may be present, to form minute, insoluble spheres of calcium carbonate. The ammonia and ammonia-like bodies as well as nitrogen, escape into the air. It is to these bodies that the urine owes its vile odor. Some of the ammonia gas remains in solution in the water or in the form of ammonium carbonate and gives to the urine its volatile alkalinity. Finally some of it unites with the urates to form the cockle burs of ammonium urate, while some combines with the phosphates to form the coffin-lid crystals of ammonio-magnesium phosphate. These various forms of crystals will be described under the microscopic uranalyses, page 109. These changes are not to be regarded as pathological unless it is evident that they have occurred before the urine was voided. In the examination of all speci-

mens this point should be determined. The presence of pus and bladder cells along with the above findings, speaks for "alkaline cystitis." To urines sent for examination to distant laboratories a single lump of gum-camphor or thymol should be added, and this advice holds especially for the summer months. In hot weather a lump of gum-camphor should be placed in the urinal before beginning the collection of the specimen. Scalding out the urinal just before the sample is collected will kill all bacteria present and delay the fermentation.

Variations Due to Diet.—The amount of water varies to a certain extent with the amount which has been taken into the body before and during the twenty-four hours the urine has been collected. The man who drinks but little is not likely to pass normal amounts of urine, and the vice versa rule holds within certain limits. The author has witnessed cases in which excessive water drinking did not dilute a concentrated urine, though it was very likely that truly pathological processes played a part in every case. A meat diet is likely to keep the urine acid, a strictly vegetable diet may render it alkaline. Diet plays a not unimportant role in the determination of the solid composition of normal urine. When the diet is excessive, there will quite likely result an increase of all the solids or certain of the solid constituents. This is easily understood when we recall that the solid matter of the urine is furnished quite as much by the decomposition products of this foodstuff excess as by the

metabolic processes. Thus there may be an increase of urea, of the urates, chlorides, phosphates and even the oxalates. In certain conditions the proteid foodstuffs when excessive, undergo fermentation in the bowel, and add to the urine, indican and other bodies which cannot be regarded as strictly normal urinary constituents. A vegetable diet is likely to increase the oxalates and carbonates, while a meat diet is likely to increase the chlorides, phosphates, and nitrogenous bodies. Certain berries have been claimed at times to influence the color of the urine, and certain foods, especially asparagus, undoubtedly contribute to its odor.

The taking of meals has considerable effect upon the urinary composition and especially upon its reaction from hour to hour; but since we are concerned mainly with the question of a twenty-four hour sample, it is certainly not worth the while to dwell upon these considerations. In the matter of alimentary albuminuria and glucosuria, see pages 44 and 64.

Variations Due to Exercise.—After continued violent exercise accompanied by profuse perspiration, the amount of urine may be reduced. This is not always true for the reason that the person is likely to drink larger amounts of water at this time. The urinary nitrogen (urea, uric acid, ammonia, etc.) is but little influenced by exercise, inasmuch as body proteids rather than carbohydrates are utilized. It is known, however, that muscular exertion will increase the phosphates.

Variations Due to Temperature.—It is observed that the amount of urine is usually increased in winter, or at least when there is a sudden change from warm to cold weather. This is explained by the fact that there is a certain amount of water to excrete, and in case the perspiration is reduced, the extra work falls upon the kidneys. These variations are not always striking inasmuch as man drinks much more water during hot weather than cold. Amorphous sodium and potassium urates are less soluble in cold than warm urine, and so they often deposit soon after the urine has been voided.

Variations Due to Medication.—The amount of urine is increased by diuretics and decreased by certain drugs as mercury when given in excess. It is also diminished secondarily by pilocarpin owing to the increased perspiration. It has been found that glucose injected into the blood will increase the amount of urine, and this may explain the polyuria of hyperglycemia. The reaction of the urine may be altered almost at will. Thus boric and benzoic acids and diacid sodium phosphate increase the acidity while the carbonates, citrates and acetates will render the urine alkaline. The odor may be altered by such drugs as asafetida, valerian, and many of the volatile oils. The urine may be darkened by the coal tar derivatives, rendered blue or green by methylene blue, or red by sulphonal, trional, antipyrin and related preparations.

Drugs may be regarded as possible sources of error in almost all the chemical uranalyses, and the

physician must by all means constantly keep this fact in mind. Thus serum albumin may be simulated by the resins, glucose may be counterfeited by decomposition products of the aromatic derivatives, diacetic acid may be confused with salicylates and indican with iodid of potash. These points will be considered along with the sources of error and discussed under the respective chemical uranalyses.

Variations Due to Preservation.—It is often necessary to preserve urinary specimens for several hours before they can be examined. This may be done very successfully, but the worker must keep in mind several sources of error in this connection. For example the author has the following incident to report. A urine which was believed to contain the indicans did not give a positive reaction. Finally it was noted that several drops of chloroform had been added and rested upon the bottom of the container. Accordingly the specimen was thoroughly mixed and other samples were examined. A prompt indican test was the result.

Formaldehyd should not be added to urinary specimens for the purpose of preservation. There are several reasons for this. It is likely to precipitate in part the serum albumin if present. It hardens and renders brittle some of the organized microscopic elements.

Complaint may be had of chloroform for the reason that it appears to dissolve some of the hyalins.

The author is of the opinion that the most excellent urinary preservative is a single lump of thymol.

Enough of this dissolves in the urine to preserve it for several days. It will not stop fermentation quite so easily as it will prevent it, so that it may be placed in the clean urinal at the beginning of the collection. Now and then enough thymol is dissolved to simulate a merest trace of serum albumin, in which case it is advisable to add an equal volume of nitric acid and heat again. If serum albumin, the precipitate will persist; but if thymol, will disappear.

Variations Due to Contaminations.—Urines may be contaminated by accident or by intention. In the former case we have usually to deal with cotton, linen or woolen fibers which may be mistaken for casts or cylindroids. Starch grains, common dirt, vegetable cells and other substances may be found when the specimen is centrifugalized, and the skilled worker may become so accustomed to ignore them that he will scarcely note their presence. Fat droplets are likely to be contributed by the smegma or catheter grease. Pus, blood, bacteria and various epithelial cells may be explained by vaginal contamination; and this is a very common source of error in the experience of the author.

The questioning must ofttimes be close in case of questioned glucose reactions. These have been noted when the specimen was submitted in syrup bottles or in tablet triturate bottles (lactose).

Hector Gavin once said, "As all animals have been classed into devouring and devoured, man holding a somewhat commanding position in the

first of these; so may human beings be considered under the two comprehensive heads of deceivers and deceived.'' Man has added almost every household article to his urine hoping to deceive the chemist. From the overworked sugar bin he has often turned to scratch the rust off the kitchen stove for uric acid crystals. Many of these attempts are crude and ridiculous, but it is well to be on the alert for them.

Sham Abrahaming is not always at the bottom of intentional tampering with the urine. Women have been known to add perfumes or even sugar to their urine before submitting it for examination to a physician with whom they were acquainted.

Variations Due to Other Causes.—The man who does considerable insurance work should satisfy himself that the sample of urine submitted has been voided by the applicant. A bottle of saffron tinted water will cause no difficulty if the specific gravity is taken. But more likely the person attempting fraud will submit a specimen from a healthy individual. In case of question it is well to complain concerning the specimen and ask that he void another into a special receptacle.

Pathological conditions may furnish specimens which may confuse the man who knows nothing of the clinical history. Thus in late interstitial nephritis, the kidneys may be passing but little save water; the author has received such specimens without instructions and has been at a loss whether to attempt a uranalysis or a sanitary examination of

drinking water. In such case it is often advisable to test for some normal urinary constituents which may be detected in very small amounts. Creatinin may usually be found by adding a little aqueous picric acid and a few drops of dilute sodium hydrate which will produce an intense red color even if creatinin is present only in one-fifth the normal amount. Rapid and repeated centrifugalization beginning with large volumes of the specimen may eventually reveal typical cells if not true casts.

CHAPTER II.

GENERAL URANALYSES.

Collecting the Specimen.—The examination of a single voiding of urine may give sufficient information in occasional instances, but as a rule it will not. The quantitative work is hopelessly impossible save when samples from a twenty-four hours' quantity are examined, because as we have shown the actual composition varies from hour to hour. At certain hours of the day, diabetic patients may pass no glucose. At other hours perfectly healthy individuals may pass small amounts of glucose. At certain hours icteric patients may pass no bilirubin, patients with valvular or kidney lesions will pass no serum albumin and patients with liver disease will pass no urobilinogen. Lung patients will pass no urochromogen, typhoid patients will pass no diazo bodies, patients with enteroptosis will pass no indican, pancreatic patients will pass no acetone. A person apparently normal in health may pass no urea for several hours; and indeed from a specimen taken at random, conclusions may be impossible, or if based upon impressions gained from such an investigation, may be dangerously misleading. The acidity varies from hour to hour. It may be quite impossible to fix a proper relation between symptoms and microscopic elements as crystals, casts,

cells and so on. It is well known that a shower of tube casts may follow the convulsions of brain tumor, but they might be absent at other times. Examination of the single specimen might lead to the diagnosis of nephritis.

Instruct the patient to empty the bladder at noon and to reject this sample. From this point he should save all urine to and including that passed the following noon. Special diets are not advisable but he should eat and drink that to which he is accustomed. In case either the vegetable or meat diet be excessive, note should be made of this as well as of any medicines he may be taking. Measure exactly the total quantity and keep record of this figure. Then mix gently the entire specimen so that the sediment may be saved. If there is but little sediment, it may be well to pour off some of the supernatant liquid before mixing. From the mixture, several fluid ounces are used for the examinations. It is best during the summer months to drop a lump of thymol into the clean urinal as soon as the collection is begun. Gum-camphor serves less well but can be used. If the specimen is sent to a laboratory for an examination, it should be placed in a clean bottle and a lump of thymol added.

Amount.—The statement that the normal urinary output varies greatly is not correct. It is reasonable to assume that the amount voided during a given twenty-four hours may be double that of the same time preceding and yet compatible with perfect health; but if the urine falls below twenty-four

fluid ounces or rises above sixty fluid ounces for considerable periods of time, it must eventually be explained upon pathological grounds, is the experience of the author. Most laboratory workers regard forty-eight fluid ounces per twenty-four hours, as an average for the person in excellent health and with good habits. We have spoken of the quantitative variations due to diet, temperature, exercise, and medicines, so let us consider what influence disease bears to the daily amount of urine. It must be constantly borne in mind, however, that all physiological factors are to be ruled out before turning to pathological explanations.

First of all is to be considered the pathological polyurias. When the polyuria is likewise a nocturia, that is, when the condition is more marked during the night, our attention is often directed toward the arterial system and the possibility of hypertension. Upon the other hand, it may be remarked that when the arterial tension is low and venous congestion exists, as in the cardiac valvular lesions, there may be a marked oliguria. The paroxysmal polyurias are doubtless of vasomotor origin, while the polyuria of diabetes may be explained by the fact that glucose is a true diuretic when it occurs in the blood in more than normal amounts. Diabetes insipidus often eludes classification from an etiological standpoint. Certain of the cases may be examples of *urina potus*, or polyuria due to excessive taking of liquids, and an investigation of the habits of the patient, will furnish

the proof. Others are undoubtedly examples of syphilitic nephritis; but some we cannot explain. A polyuria occurring in nephritis, speaks for interstitial involvement, whereas an oliguria points to parenchymatous lesion. Polyuria associated with high urea may be explained by the fact that for some reason there is an overplus of urea, and that urea like glucose, is an excellent diuretic. Such conditions have been termed azoturia.

Scanty secretion of urine when persistent, must be regarded as a very important condition, no matter what the cause, for it is not unreasonable to suppose that serious damage to the kidney parenchyma is likely when the urine is overconcentrated for long periods of time. In addition to those causes which we have considered above, may be mentioned severe fevers, diarrhea, and hemorrhages. When the urine becomes very concentrated, the precipitation of certain crystals from solution is favored. Such precipitation *in vivo*, occuring for extended periods of time, may lead to irritation, pain or hemorrhages from the urinary passages (see oxaluria dolorosa, page 110).

Oliguria may be due to decreased urea elaboration. Urea is chief of the physiological diuretics. Thus in hepatic cirrhosis, passive congestion of the liver, and so on, the stupefied hepatic parenchyma fails to synthesize urea to produce the daily three pints of urine.

Color.—When pathological coloring matters are absent, the hue varies directly with the dilution.

Normally we expect the color to approximate that of brass. In the highly diluted urines, there may be absence of color; and when the urine is highly concentrated, the color may approach that of copper. We have seen how the color may be influenced by foods and medicines.

Certain pathological pigments may impart an intense color to the urine. While the color may suggest the pigment, a final decision would not be safe without proper chemical tests. A port-wine color may suggest hematoporphyrin; a red urine, hemoglobin or methemoglobin; a black urine, melanin; a green urine, biliverdin; a golden urine, bilirubin; and a blue urine, indigo. Practically all of these colors may be given by drugs and foods, so let it be repeated, chemical proof is always necessary.

Some urines, especially those containing alkapton bodies, decomposition products of salicylic acid and carbolic acid and even melanin, turn black upon standing.

Odor.—The urine may possess no odor whatever, and this especially holds when the specimen has stood for an hour or so. When the bottle containing the sample is tightly corked, the normal odor may be preserved for days. The odor of normal urine is not unpleasant, and slightly resembles bouillon, though quite characteristic. A concentrated urine is more odoriferous as a rule than is a dilute one. We have stated that the urine may vary somewhat with the diet and with medication. The volatile aromatics to which the urine owes its nor-

mal odor have not been identified and classified. It has been claimed that one substance, urinod, may be held to account for the normal odor. In highly acid urines where hexamethylenamin is being given in large doses, a faint odor of formaldehyd may be detected. Preservatives may impart to the specimen an odor which obscures the normal urinous odor. Fermentations give rise to the stinks so commonly noted in unclean urinals. The most common of these has been considered, and furnishes ammonia and volatile ammonia-like bodies. The author has been accustomed to speak of the typical odor of ammoniacal fermentations as that resembling a mixture of ammonia and stewed turnips. At times the odor of the urine may give the impression that we are dealing with fecal fistula because of the presence of hydrogen sulphid. But it must be kept in mind that certain bacteria may break up the urinary sulphates, and no true connection need be present between bowel and urinary tract.

The redolent odors of diabetic urine have been said to resemble new-mown hay, cider, molasses and so on, but such odors are due mainly to acetone and its congenors rather than to glucose.

General Appearance.—The same cautions which have been ventured in regard to the color may be repeated at this point; viz., that conclusions from the appearance alone are unsafe, proper chemical and microscopical examinations being necessary in every case.

A urine may be almost clear when voided; but

after standing for a short time, one or more of several possible alterations occur. First of all, a flocculent deposit may collect near the bottom, and upon examination be found to consist of a network of viscid, gelatinous threads composed mainly of mucus; and suspended in the interspaces, variable numbers of cellular elements, mainly leucocytes and epithelial cells. The nubecula is more marked in concentrated urines than in dilute ones, and is more marked in females, the mucus and epithelium being contributed in large part by the vagina.

Almost any of the urinary sediments may occur in amounts sufficient to cloud the urine. In health we find the orange, pink or white urates, which may also precipitate when the standing urine cools, and be easily redissolved by heating.

The voiding of phosphatic sediments is somewhat rarer in perfect health though not impossible. The earthy phosphates may be precipitated in the urinary tract by any factor which tends either to increase the phosphates or alkalinize the urine. They may be quickly precipitated in the standing urine by loss of acidity, usually through fermentation. Unlike the amorphous urates with which they are usually confused, they are not dissolved but precipitated by heat. They go quickly into solution when the urine is rendered acid.

The presence of triple phosphates (ammoniomagnesium phosphates) in the freshly voided urine, is proof that for some reason the bactericidal properties of the lining of the urinary tract have been

lowered sufficiently to permit the growth of saprophytes and consequent ammoniacal fermentation. In some cases there may be an exception to the above rule, for a few crystals of ammonio-magnesium phosphate may occur with a fixed alkalinity. These crystals (coffin-lids or prisms) are very heavy, and sink much more quickly to the bottom of the vessel than do the amorphous urates or earthy phosphates. The phosphates are sometimes mistaken for true pus. It must be held in mind that a chemical test is not always sufficient to distinguish for the following reason. Heat and nitric acid may apparently clear a sediment and thus apparently prove phosphates. But the microscope will show that traces of pus also existed.

Uric acid is likely to occur as a brown or heavy red sediment alone or in connection with the amorphous sodium and potassium urates. It may be present even when the urine is fresh in connection with a high acidity. The crystals are dissolved with some difficulty and preserve their form as a rule so long as the urine remains unchanged. With the change in reaction coincident with ammoniacal fermentation these crystals are often replaced by the envelopes of lime oxalate.

Calcium oxalate in its various crystalline forms but usually as typical quadratic octahedra is a not uncommon sediment which may contribute to the urinary cloudiness. It may sink to the bottom of the vessel, but very frequently the crystals remain suspended in the nubecula, the entire mass some-

times rising to the surface as a scum. Fermentation plays an important role in the deposition of the crystals in old specimens. We have learned that in painful oxaluria (oxaluria dolorosa) these crystals may appear in the freshly voided urine.

Much more rarely do ammonium urate, calcium carbonate, calcium sulphate, cystin, leucin, tyrosin, xanthin, and similar sediments occur as crystals or amorphous collections in amounts large enough to give "individuality" to a urinary cloudiness, though they may accompany certain of those mentioned above. Now and then a sediment may be found, which is made up almost wholly of dicalcium phosphate rosettes (stellar, or neutral phosphates) or of ammonium urate cockle-burs. Cholesterin is very rarely found even in very diligent microscopic investigation. When cystinuria occurs, calculus formation is almost inevitable.

The urine may be clouded by pus, blood, epithelial collections, mucus, spermatozoa, fat droplets, and so on, which may be confused with the inorganic sediments or with each other. Now and then a urine may be examined in which albuminous granules or casts or both may give the specimen a distinct cloudiness. Large numbers of bacteria may impart to a specimen a dense fogginess.

Specific Gravity.—The specific gravity varies normally between 1012 and 1025, being high or low in health according to the concentration of the urine. In apparent health the specific gravity may rise above or fall below these limits. In such case other

examinations should be made, for it is very likely that such readings when persistent, are incompatible with health. That is to say, a specific gravity persisting below 1010 in a mixed twenty-four hour specimen, should suggest careful inquiry as to the amount of urea and so on; or when the specific gravity fails to fall below 1030, may suggest a lack of water in the urine or overplus of some one or several constituents, the latter possible strictly pathological. In diabetes insipidus and in interstitial nephritis there is a persistent polyuria with a low specific gravity; while in diabetes mellitus and azoturia, there is likewise a persistent polyuria, but a high specific gravity. In parenchymatous disease of the kidney the specific gravity is high, while the total amount per twenty-four hours is likely to be reduced. When high specific gravity occurs without polyuria, there is a tendency upon the part of the difficultly soluble salts to precipitate out, and such precipitation can be prevented only by other factors as excessive acidity and so on.

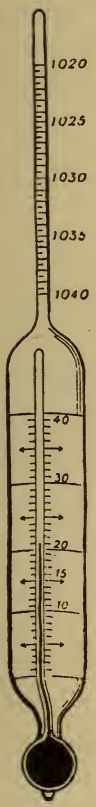


Fig. 2.—Urinometer.

For routine work temperature corrections are

scarcely necessary if the technic is carried out with specimens which have neither been cooled nor heated.

For the specific gravity estimation we use the urinometer, a special hydrometer. The following cautions must not be overlooked when estimating the specific gravity:

1. The cylinder should not be filled over four-fifths or some of the urine will be displaced by the urinometer.

2. If foam is present on surface, remove by a piece of filter paper or blotter before placing the urinometer in the cylinder.

3. The urinometer must not come in contact with the inner surface of the cylinder but must float free near the center of the sample. Contact with the cylinder may be avoided to some extent if the urinometer be given a slight twirl just as it is placed in the urine.

4. Make reading from the lower meniscus.

In case the amount of urine is small and a specific gravity reading is considered more desirable than other examinations, the sample may be diluted with distilled water and a reading taken. The last two figures of this reading multiplied by the dilution will give the correct specific gravity.

Reaction.—The working methods of the various laboratory authorities vary so hopelessly in respect to selection of indicator, classification of bodies to which reactions are due, clinical significance of find-

ings and so on, that this book cannot hope in its available space, to attempt a review or reconciliation, but merely to give in brief, a resume of the views and working technic employed by the author. It is well to warn the reader that while some of our workers have given too little attention to the reaction of the urine, others have perhaps gone too far. A large amount of information concerning the diagnostic and therapeutic problems of reaction have been omitted in order to emphasize essentials.

Certain workers have shown that free organic acids are present in the normal urine, or at least in some apparently normal urines; but the observation is doubtless inconsequential so far as clinical purposes are concerned. The normal urinary acidity is due almost entirely to certain acid salts especially diacid sodium phosphate, and such traces of free acid as may be detected by the sensitive (not clinical) methods, may be ignored. At any rate it is true that neither uric acid nor acetone series contribute to the normal urinary acidity.

While single specimens of normal urine may be alkaline, it is rare that the mixed twenty-four hour excretion is ever alkaline. If such is the case, an inquiry concerning the diet will usually provide an explanation. With phenolphthalein (an indicator which like all others has its shortcomings, but which is best for accurate work) practically all normal urines are acid.

Urinary hyperacidity which cannot be explained by diet or medicines must always be regarded as

pathological. Most commonly this hyperacidity may be explained either by the presence of certain microbes associated with the putrefaction of proteid foodstuffs (usually in colon) or associated with the decomposition of body protoplasm. Into a third class fall those hyperacidities due to vicious katabolic processes in the body cells. In the first case, we have to deal with intestinal toxemia, a process but partially understood; in the second with true infection in tissue or organ and which may be located with difficulty even when suspected; and in the third, with diseases of metabolism especially the acidoses of children and true diabetes. In the first case we have to deal with scarcely pathogenic bacteria which produce acids and acid-like bodies by excessive and erroneous putrefaction of proteid foods, and such acids can occur in the urine only after absorption from the bowel.

The fact must not be lost sight of that a urine may not be hyperacid when secreted by the kidney but become so in infections of the urinary tract. Thus in colipyelitis or in tuberculous kidney, it is not unusual to find the so-called "scalding urines." Now and then we have to deal with hyperacid urines where an explanation seems impossible, as for example in the uricacidurias where excess acidity is coincident to (though not caused by) uric acid.

Estimation of Acidity.—For practical purposes it is not necessary to carry out the various estimations of mineral and organic acids. We are rather inter-

ested in the question of total acidity to determine if possible whether the specimen is or is not excessively acid. The titration methods are used in the larger laboratories where much of this work is to be done. For the occasional estimation, the method of Harrower is both simple and fairly accurate.

Instead of adding the alkali from a graduated buret, the urine and indicator are placed in a graduated test tube and to this the alkali is added drop by drop from a pipet.

The urine is poured into the acidimeter until the lower meniscus reaches the 10 c.c. mark. A few drops of phenolphthalein are added. Then by means of a pipet or medicine dropper, decinormal sodium hydrate solution is added drop by drop. After each drop is added the tube is shaken or inverted so that the contents are thoroughly mixed. When the first permanent pink occurs, the acidity is read off in degrees as marked by the lower meniscus. The normal acidity of a mixed twenty-four hour specimen averages between 30-40 degrees. Estimations must of course be made **only from mixed twenty-four hour specimens** which have been kept sweet by refrigeration or preservatives. For we have shown that the reaction of the urine varies somewhat from hour to hour and is altered by urine decomposition. An acidity of 50 degrees may not be strictly overplus, but anything above that must be regarded as pathological.

Pathological Hyperacidities.—Briefly these may be classified:

1. *Copremic Hyperacidities.*—This has been discussed. We are not certain as to the exact nature of all the acids. Among them have been found indolacetic acid, unknown acids containing sulphur, paraoxyphenylacetic acid, paraoxyphenylpropionic acid and so on. Likewise we may find the indicans (red or blue), phenol, paracresol and similar bodies. In the opinion of certain workers, much of the ammonia which normally goes to form urea may be used instead to neutralize the acids that the fixed alkalies of the tissues may be spared. At any rate the urea may be low without reduction of the nitrogen, and examination will show a high ammonia. It is not in the scope of this book to consider the symptomatology of copremia. We have come to believe, however, that long continued acidemia* must be an important cause of nephritis. Indeed we have found that traces of serum albumin and hyalin casts are quite likely to accompany hyperacid urines and suggest nephrosis. Moreover Martin Fischer and others have shown that acid retention in the renal parenchyma, is an important factor in the retrograde changes and desquamation of the ultimate secreting kidney unit.

2. *Pyemic (Infectious) Hyperacidities.*—The pathological process is similar if not identical with the above. In the manufacture of the poisonous acids, tissue protoplasm rather than proteid food-

*The use of the term "acidemia" has been challenged (Jour. Am. Med. Assn., Vol. lxvi, No. 5, p. 376). It has been used in this connection, not to indicate the reaction of the blood, but of the urine, and for want of a better term.

stuffs are utilized. The lesion may be but a simple nephrosis, but we have good reason to believe that eventually a point may be reached where hopeless and progressive nephritis results. The focus of intoxication may be easily found in some cases. More likely we are dealing with a low grade of chronic infection hidden away, and local symptoms may be absent. Thus purulent and necrotic processes may be concealed in the nasal sinuses, teeth, tonsils, lymph glands, old lung cavities, gall bladder, appendix, prostate gland, and so on.

3. *Hyperacidities of Acidosis.*—Recently it has been learned that acidosis may occur without true diabetes, especially in children, after anesthesia and so on. Betaoxybutyric acid, acetone, diacetic acid and other unknown bodies may be present in the urine.

4. *Local Hyperacidities.*—A urine when secreted may be normal in reaction but become hyperacid before reaching the meatus urinarius. Where excessive acidity is due to bacteria, the renal parenchyma doubtless escapes injury from such acids. It is well known that in renal phthisis, the urine may become very highly acid before pus appears. In colon bacillus infection of the renal pelvis, acid formation is quite the rule. It would appear from the investigations of the author that now and then we have to deal with urinary hyperacidities due to unknown fermentation processes. This is seen in the beneficial influences of urotropin in some of the painful oxalurias associated with excessively acid urine.

5. *Cryptogenic Hyperacidities.*—High acidity often accompanies the presence of uric acid crystals in the urine. It is also due to unknown processes of fermentation in the urinary tract. At least we believe that uric acid does not of itself contribute to the acidity of the urine, but that the whetstone formation is consequent.

Pathological Hyperalkalinities.—A persistently alkaline urine (twenty-four hour mixed specimen examined when fresh) cannot be regarded as physiological:

1. *Fixed Hyperalkalinities.*—Persistent alkalinity which is not due to ammonical fermentation may usually be explained by medication or diet. We believe that vicious katabolic processes are never alkaline but invariably acid producing. The absorption of large exudates or transudates has been claimed to have rendered the urine alkaline.

2. *Volatile Alkalinity.*—This state is doubtless always pathological. A freshly voided specimen must be examined. High ammonia with low urea and no pus or symptoms of cystitis may point to hepatic lesion. However the damage to the liver parenchyma may be very great without disturbances of the urea function.

The term volatile alkalinity is usually reserved for the urines of those diseases of the bladder accompanied by partial urinary retention or other condition favoring fermentation of an ammoniacal type. Pus, blood, vesical cells, and hosts of bacteria usually are found.

CHAPTER III.

CHEMICAL URANALYSES.

It may be stated that as a rule the urine should be filtered before undertaking the chemical tests. There are exceptions, especially where a single test for glucose or serum albumin is made upon a clear, freshly voided urine. The rule holds very rigidly, however, in case the urine is not perfectly transparent. In the albumin tests, clear and untreated samples should always be set up as controls unless the worker has considerable experience in detecting traces.

A clean glass funnel and clean filter paper are used. In some instances it may be impossible to clear the urine by filtration, even though several thicknesses of filter paper are used. In case this cloudiness is due to bacteria, it may be impossible to decide whether serum albumin is or is not present. The urine should be shaken with calcined magnesia and filtered or recourse be had to other samples. But if the cloudiness is due to earthy phosphates or amorphous urates, the heat and nitric acid test will clear this up. Any urinary cloudiness which cannot be removed by filtration, and which cannot be explained by the presence of hosts of bacteria, is in all likelihood due to these inorganic sediments, and when testing for serum

albumin, the heat and nitric acid tests will decide.

Euglobulin.—To 5 c.c. of clear urine, are added about 20 c.c. of cold distilled water and then a drop of glacial acetic acid. Set up alongside a control of clear, untreated urine. The mixture may be shaken but not heated. After a short time a cloudiness may be noted. This is due to euglobulin (nucleoproteids).

When a slight albuminuria (as determined by the heat and nitric acid test) proves to be a euglobulinuria (as determined by the above reaction) it is very likely that we are not dealing with true serum albumin. This point should be held in mind when testing for the trace of albumin.

There is a tendency upon the part of clinicians to regard euglobulinuria as a benign albuminuria and significant of true body defenses; i. e., combinations of neutralizing proteids with acids (nucleinic, sulfuric, taurocholic, etc.); and they are excreted by the kidney as waste products of metabolism. Often we are able to dispose of the diagnosis of possible nephritis by means of this test, although such a disposal is not made by life insurance companies who look upon all albuminurias as bad risks. Many if not all of the functional albuminurias are euglobulinurias, and as will be shown later, all of the serum albuminurias are distinctly pathological. The trace of albumin accompanying excess of indican, is usually euglobulin and may be explained by the excess of organic acids accompanying the indican, although serum albumin sometimes may be found in

indicanuria and here suggests actual damage to the renal parenchyma. Euglobulin is sometimes found in apparently healthy individuals and in the absence of other urinary changes, but euglobulin though not to be taken as evidence of renal lesion is perhaps at least semipathological. Euglobulin is suggested when an excess of the nitric acid clears up a "hesitating" cloudiness.

At least one source of error must be kept in mind when applying the euglobulin test. In diseases of the renal pelvis, ureter and bladder, there is an increase of the nucleoproteids; and the reaction to acetic acid may closely simulate euglobulin. In the presence of such lesions and especially when pus is present, the test cannot well be applied.

Recently Pollitzer seems to have advanced a step further in the explanation of some of these acetic acid reactions. It has been known that chondroitic acid when added to the urine along with a drop of acetic acid will coagulate serum albumin. Upon the other hand chondroitic acid may occur in urines; and in the presence of serum albumin even though in traces, may be precipitated out by acetic acid. Furthermore if serum albumin is not present in the sample, by adding small amounts along with the acetic acid, large precipitates may occur in the night urines of orthostatic albuminurias, urines of tonsil infections, and so on, all of which have been shown to contain other organic acids. It is likely if Pollitzer's observations are confirmed, that we will ar-

rive very closely to an explanation of certain albuminurias as yet but little understood.

Serum Albumin, Significance.—Serum albumin, or true albumin must be regarded as a pathological finding, at least when it occurs persistently in the urine. Its diagnostic and prognostic significance depend upon other urinary findings and upon other clinical data, rather than upon the actual amount which may be present from day to day. The author has observed cases in which serum albumin has occurred in rather large amounts for years. These patients are enjoying relatively excellent health, but of course not perfect health or prospects. Upon the other hand mere traces have been found in the rapidly fatal cases of Bright's disease. Clinically we adhere to old classifications, mainly because every albuminuria cannot be properly placed by the pathologist. From the standpoint of the latter, the following classification would be excellent were we but able to place each given albuminuria into its proper class or to tell just when, for example, an albuminuria of the first classes becomes an albuminuria of the C or D type.

Class A.—Albuminurias due to overplus albumin content of the blood including the alimentary albuminurias. It seems probable that there are many types of serum albumin, and that the amount in the blood need not be especially great, but the proteid be abnormal in composition, so far as the needs of the body are concerned, for excretion to occur.

Class B.—Albuminurias due to disturbances of nutrition in the capillary tufts (endothelium) or in Bowman's capsule (epithelium) but no permanent injury. Thus in the albuminurias associated with vascular hypertension or hypotension, the febrile and milder toxic albuminurias we may have an explanation.

Class C.—Nephrotic albuminurias, true renal albuminurias where autopsy will later demonstrate actual lesion, but where this lesion is a nephrosis, or the retrograde and reparative changes consequent to the action of some poison either chemical or infectious. These albuminurias are likely to yield if the injurious focus of poison manufacture be removed or the poison be neutralized.

Class D.—Nephritic albuminurias, true progressive renal albuminurias where autopsy will later demonstrate actual renal lesion, but where this lesion is a definite, classical nephritis rather than alterations consequent to the poisons from some distant focus. Either of the foregoing albuminurias may become a nephritis albuminuria.

This volume cannot delve deeply into a consideration of the many forms of albuminuria. The significance of any albuminuria, let it be repeated, depends not so much upon its intensity as upon other clinical data, especially upon other urinary finds. Below is given an outline which may aid somewhat in diag-

nosticating a renal albuminuria, but it must be kept in mind that we are not always able to say whether or not the process is progressive even with bedside data:

1. To prove a renal albuminuria, it is necessary that the albumin be serum albumin.

2. Such an albuminuria must be persistent or nearly so. In vascular nephritis it may be intermittent and only frequent tests will show it. Diuresis often explains why renal albuminurias may apparently cease, the clinical trace being rendered occult by dilution.

3. If possible, other types of albuminuria should be ruled out. Inquiry should be made into the possibility of euglobulinuria, Bence-Jones albuminuria, fever, hepatic and cardiac disease as well as tuberculosis of the kidney and other local conditions.

4. The serum albumin should be backed up by other urinary findings; viz., casts especially cellular or granular types, urea paucity, renal cells, red blood cells, fatty granules, and protoplasmic debris.

Serum Albumin, Detection.—Serum albumin occurs in the urine as a sol., or colloidal solution and there seems to be almost no limit to the quantity which may be present in a small amount of the urine. In one case examined by the author, a perfectly transparent urine when heated became an amorphous mass from which but a few drops of water could be secured.

The protein color reactions are not specific inasmuch as other albumins or near-albumins may oc-

cur in urines. For the identification of serum albumin we have recourse to the coagulation tests, the resulting gel (incorrectly termed, precipitate) being visible to the eye as a cloudiness, coagulum or putty-like mass depending upon the amount of serum albumin present.

A very large number of coagulation tests have been proposed, and it may be stated at the outset that there is no single "all-round" test known. Three tests have been described below. The heat and nitric acid test should be used routinely and especially with those urines not easily cleared by filtration, where the question of Bence-Jones body, albumoses, urates, and phosphates enter, and in all urines which fail to show a cloudiness with other tests. Though usually regarded as a comparatively coarse method, it will often show serum albumin where the other tests fail.

The other tests are recommended where it is desired to prove a trace of serum albumin. They cannot be applied to clouded urines, neither are they applicable when the possible presence of other protein bodies plays a part. The sulphosalicylic acid test is regarded as the most sensitive of all clinical methods.

Heat and Nitric Acid Test.—Boil a specimen of the filtered urine. The sample should fill a test tube half full. If such cloudiness as has been present disappears, it has been due to amorphous sodium and potassium urates. If the urine is scarcely acid, a precipitate of earthy phosphates may occur which

will resemble very closely the cloudiness due to serum albumin, but which will be dissolved by adding acid. When the urine becomes hot but does not boil, observe it closely for evidences of cloudiness. (In alkaline urines serum albumin in small amounts may not be precipitated by heating, and for this reason it is often advisable to add just enough acetic acid at the beginning to neutralize the alkalies.) When it is certain that the cloudiness cannot be cleared by moderate boiling (see Bence-Jones body, page 53), a couple of drops of pure nitric acid may be added. If the deposit does not disappear, we may be dealing with either serum albumin or euglobulin (the latter may account for the cloudiness as shown by the euglobulin test) or by adding an excess of the nitric acid the cloud will vanish. If but a few drops of the nitric acid clears the urine it is probable that the cloudiness was due to earthy phosphates, or, less likely, oxalates. An effervescence upon adding the acid, may be explained by the evolution of carbonic acid gas and free nitrogen by the breaking up of the carbonates. In case the urine turns brown or mahogany in color we are dealing with the indicans, bile pigments, iodids or aromatic medicaments or other pigments. The sources of error will be considered below (see page 50).

Nitric Acid Test.—This is often described as the cold test. The urine must be clear and Bence-Jones body be ruled out by other tests. This method has been very popular with clinicians and has been

variously modified but the principle is the same—floating a layer of the urine on a layer of the nitric acid. Heller's test is carried out by carefully pouring the urine down the side of a test tube previously filled up to 5 c.c. with the acid. Simon prefers a conical sedimentation glass for the test. Boston uses a glass pipet permitting the urine first to enter, and then sinking the pipet beneath the surface of the acid in such a manner as to secure a lower layer of this. A medicine dropper may be used in a similar manner.* The nitric acid must be pure and concentrated, not fuming, as pigment reactions may interfere with the test. At the junction of the urine and the acid, a distinct white ring forms, and this is made up of the albumin coagulum.

Sulphosalicylic Acid Test.—Although the nitric acid test is regarded by many as the best method for detecting the trace of serum albumin, the author has used sulphosalicylic acid for this work with much better results. Though somewhat more costly it possesses the following advantages over nitric acid:

1. Much safer to handle.
2. May be kept for long period of time. After a certain length of time nitric acid begins to fume.
3. More sensitive reagent for serum albumin. In the author's laboratory it is not uncommon to find serum albumin in many urines which fail to give a cloudiness with nitric acid. The sulphosalicylic acid test is, however, an accepted clinical

*Williams: Laboratory Methods, page 111.

test and will not show the minutest traces of serum albumin regarded as physiological. The reaction is of especial value where casts and other evidences of disease are found, but where albumin is not demonstrated by other tests.

The urine must be clear. It must contain no appreciable numbers of bacteria and no granules either of inorganic sediment or disintegrated cell protoplasm, points easily determined by microscopy and other tests. A strong but not concentrated solution of the reagent in distilled water answers the purpose very nicely. A couple of drops of this solution are added to 5 c.c. of the transparent urine, and shaken sufficiently to mix. Do not heat. If after a couple of minutes, no cloudiness is noted, add two more drops. Compare with untreated sample of the urine as control.

Serum Albumin, Sources of Error.—Most of these have been considered. It is best when deciding the questionable cases, to use the heat and nitric acid test. In case bacteria are present in large numbers and the urine cannot be clarified by filtration, the cloudiness cannot of course be cleared up by heat and nitric acid. Microscopy explains the trouble, but still in cases where it is desired to demonstrate the trace of serum albumin, there may be the opinion that such bacteria as are present cannot explain all of the cloudiness (contrast controls). Or if there is further question and it is impossible to secure a sample free from hosts of bacteria, shake up another specimen with calcined magnesia powder

and filter. Reject the first portion of the filtrate and test the last portion. This will rid the urine of most of the bacteria. Urines containing blood and pus will contain serum albumin, and tests cannot be set up with diagnostic profit even though the urine has been filtered, for only the cells remain on the paper and the albumin of the pus or blood as well as that derived from the kidney disease pass through. Whether blood or pus are actually present may be proved only by microscopy, and it is folly to conclude that a positive albumin test means nephritis, if microscopy is neglected. If the number of blood or pus cells is small and large amounts of serum albumin are present in the urine, it is not reasonable to assume that all of the latter may be explained by the presence of the former. It may be very evident that some of the albumin is kidney albumin.

If there is question in regard to the presence of Bence-Jones body, protract the boiling and do not add the nitric acid. In the case of possible euglobulin, add an excess of the nitric acid or test another sample of the urine directly for euglobulin. Both serum albumin and euglobulin may occur in a specimen of urine, but this is rarely noted when pus and blood are absent. In such cases set up contrast tests alongside.

Medicinal resins (copiaba, santal oil, etc.) do not precipitate readily during the heating but the cloud is formed when the acid is added. In questionable cases where euglobulin and earthy phosphates can be ruled out, it will not be necessary to add the acid,

but the boiling may be continued. Unless a history of medication is at hand the question may be a difficult one to decide, if the urine is alkaline, for the necessary acidulation will favor either an albumin or resin cloud. Advice is sometimes given to permit cooling of the specimen and add alcohol to excess which will clear the liquid if the cloudiness be due to resins. But we have learned that perhaps some of the serum albumins may likewise disappear with such treatment. Briefly, therefore, a history of the medication is almost imperative. In case the physician sets up his own tests, he should not be misled. If he submits the sample to a laboratory man, he should supply this information.

Finally it must be said that in exceptional instances, many other substances may simulate serum albumin—the bile acids, urea (as urea nitrate in concentrated urines), uric acid, gypsum, and perhaps others. In questionable cases it is well to turn to the suggestion of Winternitz. Remove the questionable coagulum or precipitate from the urine, and then thoroughly wash with hot, distilled water. When the filtrate ceases to give with silver nitrate a marked cloudiness, it is probable that all urine has been removed. Now pour upon this washed material in the filter, several drops of boiling Millon's reagent. A red color shows the protein nature of the material.*

*This reaction is due to the aromatic nucleus of the protein molecule. The urine must be entirely removed as similar reactions may be given by albumoses, tyrosin, indicans, and so on. Millon's reagent is made up by dissolving one part of mercury in one part by weight of concentrated nitric acid. The mixture is left cold for a

Bence-Jones Body.—This was once believed to be an albumose but it has been shown to be a true albumin. It is almost pathognomonic of certain bone tumors especially of the myelogenic osteosarcomata. It has, however, been identified in the urine of leukemia and the author has found it in one case diagnosed as neurasthenia in which no organic disease was discovered. The simple heat test is applicable for its detection. If the urine is alkaline, add a drop or two of acetic acid. Heat 5 or 10 c.c. in a test tube. As the urine becomes hot a coagulum rapidly forms but disappears when the boiling point is reached, finally to reappear when the contents of the tube are cooled under the tap. The reaction is characteristic and is not simulated by the other protein bodies, the latter when heated to 60° C., coagulating irreversibly or not at all.

Globulins.—Serum globulin and related bodies are coagulated by all of the clinical tests for serum albumin. By special methods they may be differentiated from serum albumin, but inasmuch as they occur along with it and have no known diagnostic significance in themselves, space will not be given to the discrimination.

Noncoagulable Urinary Proteins.—Peptones, albumoses and perhaps other bodies are included in this class. Urine from which the coagulable proteins have been removed by heat and filtration

few hours and then gently heated until solution is complete. Then add two volumes of distilled water. Allow to stand from twelve to twenty-four hours and finally decant the clear liquid from any crystals which may have settled.

(making certain that the specimen is acid before attempting this procedure), may be tested by Millon's reagent. In the author's laboratory, Fittipaldi's test has given considerable satisfaction. To 5 c.c. of urine are added 30 c.c. of absolute alcohol. On the following day the liquid is decanted and the coagulum dissolved in a small quantity of soda. On treating this liquid with a freshly-made, ammoniacal solution of a nickel salt, an orange-red tint shows the presence of albumose. The clinical significance of the noncoagulable proteins is not always clear. They may be found in severe infectious fevers and in cachexias. The finding of albumose in considerable quantity has been claimed to be pathognomonic of fetal death; and in questionable cases it has been advised that the urine of the mother should be tested. However this contention has not been conclusively proved.

Many other protein bodies have been reported as being found in specimens of urine; but the diagnostic meaning of these has not been made clear. Amino acids have been found in normal urines. In acute yellow atrophy of the liver, this organ fails to convert the excess of amino acids into urea and they may be found in the urine (see leucin and tyrosin, page 113).

Hemoglobin and Related Bodies.—Two types of hemoglobinuria must be distinguished; viz.:

1. *True Hemoglobinuria.*—This occurs in connection with hemoglobinemia (release of hemoglobin from erythrocytes in the blood stream).

Hemoglobinemia sufficient to result in hemoglobinuria, is caused by certain poisons (chloroform, chlorates, crotalin, carbolic acid) called hemolytic bodies. Even quinin and the coal-tar antipyretics may lead to hemoglobinemia and consequent hemoglobinuria if administered in large doses. Upon the other hand hemoglobinemia may be observed without hemoglobinuria as in chlorosis where there is rapid loss of hemoglobin, we may not be able to demonstrate it in the urine of the patient. In true hemoglobinuria few or no red blood cells are found in the urine.

2. *Hematuria, or Incidental Hemoglobinuria.*— Hemorrhage may occur at some point in the urinary tract, as in the painful oxalurias, renal calculus, the classical nephritides, renal phthisis, renal cancer, almost all of the organic diseases of the bladder and prostate and even from the urethra. By microscopy the erythrocytes may be identified, though sometimes with difficulty if the hemorrhage occurs high in the urinary tract or if there is retention in which case crenated cells and blood shadows may take their place. But by careful work, true hemoglobinuria and hematuria may usually be distinguished. The two conditions may of course coexist but the study of many urinary specimens shows that they do not as a rule occur at the same time.

Hemoglobin and its derivatives cannot be considered normal urinary pigments. The finding of these bodies by chemical tests, proves organic disease, but the finding of red blood cells is almost nec-

essary before we can prove that this blood was released in the urinary tract. Furthermore the physician must be on the lookout for possible contamination from the genitals, especially in menstruation; and in questionable cases catheterization may be imperative for the distinction.

Known that the blood comes from the urinary tract, it often becomes very necessary to determine the exact point of the bleeding. Much appears in medical literature upon this subject, but it is not always an easy matter. The modern cystoscopic work promises more than do either bedside or laboratory examinations. This being a laboratory book, a few points will be considered as regards the location of the lesion by the uranalysis.

1. Kidney blood is likely to show more crenated forms and ghosts than where the hemorrhage occurs lower down. Other renal findings as casts (especially blood casts), renal cells and so on may give the clew. The coexistence of an acid pus is likewise suggestive.

2. Ureteral blood may be accompanied by ureteral cells and in some cases by large numbers of minute acicular crystals of lime oxalate.

3. Bladder blood is not flaky as a rule unless retention renders it so, and retention may always be proved clinically. When alkaline pus coexists, it points to bladder origin for both pus and blood. Vesical cells have a similar significance.

Detection of Hemoglobin and Derivatives.—Three excellent tests are given below. The first of these

is practically specific for blood pigments. The other two are by no means specific being given by an almost endless list of other substances, but are detailed because they are extremely sensitive and when absent, rule out all possibility of the presence of hemoglobin.

Heller's Test.—Strongly alkalinize 10 c.c. of the urine with sodium hydroxid. Heat to boiling but do not boil. A precipitate may be explained by the earthy phosphates and other salts. If however this precipitate is colored red, we know that hematin, a hemoglobin decomposition product, is present. This is not a very sensitive test as contrasted with those to follow, but is practically specific.

Van Deen's Test.—Keep in mind the interpretation noted above. Mix thoroughly 5 c.c. of urine and 5 c.c. of a freshly made tincture of guaiac (made by dissolving a piece of gum guaiac in absolute ethyl alcohol). The mixture will become milky in appearance. Add 2 to 5 drops of pure peroxid of hydrogen and shake once more. Set aside the tube. At once or within a short time a blue or green tint will be noted which may be counted a positive test. Alkaline samples must be acidified with acetic acid before applying this test.

Aloin Test.—Keep in mind the interpretation noted above. To 5 c.c. of the urine, add 1 c.c. of glacial acetic acid. After 30 minutes add 3 c.c. of ether and cork to prevent evaporation. After 30 minutes, pour off this ether into a small test tube

or a clean vial and add a grain of powdered aloin. Finally add an equal volume of pure peroxide of hydrogen. A positive test is shown by a cherry-red color.

Bilirubin and Related Bodies.*—Even as hemoglobinuria may be noted in hemoglobinemia, so may bilirubinuria accompany bilirubinemia (by bilirubinemia in this connection is meant the presence of bilirubin in the blood and tissues). We cannot enter into the various causes of bilirubinemia and cannot consider in this volume the clinical manifestations of the condition, but must give our attention chiefly to the chemical derivation of bilirubin and why it is found in pathological urines. Waste hemoglobin is excreted by the liver cells, and passes from the liver capillaries normally as bilirubin (hemoglobin minus iron and globulin); and bilirubin is a poisonous substance. Normally this bilirubin and its secondary products pass into the intestine, are further altered into less poisonous bodies, and escape from the body by the bowel and urine.

But in certain pathological processes, it is not completely excreted by the biliary capillaries, but passes back in part unaltered into the general circulation to be eventually deposited in the tissues or excreted—mainly by the kidney. Once a part of the hemoglobin molecule and favored in chemical, physiologic processes, bilirubin has been robbed of

*Williams, B. G. R., "Bilirubinuria," Illinois Medical Journal, February, 1912.

its iron and cast aside as a waste substance. Entering again into its former domicile, it is made rid of by the circulation as rapidly as possible and at any cost. Possibly it is neutralized in part, but that which is not thus disposed of destroys many cells. Among these cells, the following type bear the chief injury.

1. Hepatic cells, leading often to urobilinogenuria or decreased urea elaboration.

2. Erythrocytes, leading to erythrocytolysis and even hemoglobinuria.

3. Blood platelets or other cells and substances concerned in blood clotting. In bilirubinemia, the coagulation time of the blood may be greatly lengthened.

4. Renal cells, toxic desquamation into uriniferous tubules. Renal cells and albumin often accompany bilirubin in the urine.

Bilirubinuria, even though slight, must be regarded as a pathological condition.

Tests for Bilirubin and Related Bodies.—Though arising from hemoglobin, these bodies cannot be identified by those methods which we use for hemoglobin and its other derivatives.

In marked bilirubinuria, the urine may be golden, but there are other golden urines, and chemical tests should always be applied. The presence of yellow or green tinted casts in a specimen should always lead us to test for bilirubin.

Foam Tests.—Shake very thoroughly a sample of the urine. A greenish, yellowish or brownish tinge

shows that bilirubin must be present. No known urinary pigment will so color the foam though of course extraneous substances introduced into the sample might do so. This test may be confirmed by the chemical reactions.

Filter Paper Test.—To a large quantity of the urine (acidified if alkaline) add aqueous solution of barium chlorid, drop by drop until no more precipitate appears. Mix well the contents of the tube, and filter several times through a thick filter paper. The bilirubin is held on the paper with the sulphates and phosphates. Meanwhile to 5 c.c. of concentrated nitric acid in a test tube, add a bit of clean wood (tooth pick or match stick) and heat carefully until yellow fumes are given off. Then cool under the tap. When quite cool, this reagent will be ready for use.

After filtration has been repeated and complete, remove and unfold the filter paper. Dry off excess liquid with another filter paper very carefully so as not to disturb the precipitate. Permit a drop of the cool, yellow acid to fall upon the paper. In the presence of bilirubin, a green band forms at its edge. Rapidly this band journeys outward and is followed by other color rings—blue, indigo, purple, red and yellow, but the green ring is always external and is the specific ring for bilirubin, as indican, iodids and other substances may give rise to the others. This test is doubtless much more specific than the foam test, but not always so sensitive, as very small amounts of bilirubin may be appre-

ciated with the foam test by the experienced observer.

Hammerstein-Nakayama Test.—This is a very sensitive test for bilirubin. Equal parts of an acid or acidulated urine and ten per cent barium chlorid solution in distilled water are mixed and well shaken. Ten cubic centimeters of this mixture are placed in a centrifuge and centrifugalized until the supernatant liquid is clear. Reject the liquid and keep the sediment for the testing. Now quickly make up the Hammerstein reagent as follows:

Acid nitric, 25%..... 1 drop.
Acid hydrochloric, 15%.....19 drops.
Alcohol, 75% ..... 5 c.c.

About one-third of this reagent is quickly added to the precipitate in the centrifuge tube and the contents shaken thoroughly. Centrifugalize. A green tint in the supernatant liquid indicates the presence of bilirubin.

Iodin Test.—A few drops of tincture of iodin are superimposed upon 10 c.c. of the urine. A green contact ring appearing within a minute is due to bilirubin. A greenish ring appearing later is not regarded as a positive test.

Bile Acids.—Until the past few years the bile acids or their salts were supposed to be the toxic and otherwise important causes of those morbid symptoms coincident with the entrance of the hepatic excretory products into the blood. Their detection has always been a difficult matter. It is

with considerable relief, therefore, to the diagnostician that these older views have been proved incorrect and his attention directed to bilirubin. For this reason, a consideration of the bile acids has been omitted and the reader is referred for particular information to books upon physiological chemistry.

Urobilinogen, Its Significance.—The urobilinogen reaction may be regarded as a functional liver test. Normal liver cells do not permit the escape of urobilinogen of appreciable quantity into the general circulation and into the urine. A very slight injury of the liver may be followed by the presence of urobilinogen in the urine. It seems that uninjured cells of the liver are able to compensate in part for those which have been distroyed or weakened. Thus urobilinogenuria may be present in a mild condition where most of the liver cells are involved; for example, passive congestion, angiocholitis, cirrhosis, hepatic syphilis and so on; it may not be found in certain of the local severe diseases of the liver, as abscess, cancer, or trauma. The disease need not be primary in the liver for urobilinogenuria to occur. The condition may be secondary by virtue of poisons, anemia, congestion, and so on, leading to stupefaction of the parenchyma. It is commonly noted in the venous congestion of valvular lesions, in arterial disease, in pernicious anemia, typhoid, and scarlet fever. The test is a very sensitive one, and is not a measure of the severity of the lesion. Urobilinogenuria occurs early in all condi-

tions resulting in stupefaction and sluggish functionation of the liver cells; amino acids appear in the urine when the cells are undergoing actual destruction.

Urobilinogen, Its Detection.—The urobilinogen reagent of Ehrlich and Neubauer, is prepared as follows: Dissolve one gram of paradimethylaminobenzaldehyd in 10 c.c. of pure hydrochloric acid. Then add 5 drops of alcohol and distilled water up to 50 c.c. total quantity. This may be kept for a considerable period of time.

To 5 c.c. of the filtered urine add a couple of drops of the reagent and shake gently. Do not heat but set aside at room temperature. After a minute or so the presence of urobilinogen will be shown by the appearance of a beautiful cherry red color. The reaction may be delayed an hour or so in some cases but usually appears in five minutes. A yellow or pinkish tint (observed by holding the tube in direct sunlight) must be regarded as a negative reaction.

Uric Acid, Its Detection.—Uric acid appears in the urine as such or as its salts. It may be found in normal urine, but when present in considerable amount must be regarded as pathological or semi-pathological (may be greatly increased by a heavy proteid diet). It is well to be able to identify it as a constituent of calculi, and so the following test is given: To some of the material, add an equal quantity of nitric acid and evaporate in a porcelain dish on a water bath until quite dry. A yellowish residue remains. Remove the dish from the bath and

permit it to cool. When quite cold, expose to ammonia fumes. The residue becomes reddish-purple if the material is uric acid.

Glucose, Its Significance.—Traces of glucose may be identified by special methods in practically any specimen of urine. Such a glucosuria is termed physiological. Considerable amounts of glucose may appear in the urine but be transient and the case is not one of true diabetes. To this condition is given the term, alimentary glucosuria. The normal limit of sugar assimilation varies not only in different persons but at different dates in the same person. To prove diabetic glucosuria, it is necessary to prove that glucose is persistent or almost so even when carbohydrates are somewhat restricted. In some atypical cases of true diabetes, glucose may be present in the urine for a few days or weeks, disappear when the diet receives but slight attention only to reappear with the slightest excuse. Some of these baffling cases may be affected with diabetes for many years, be complicated with diabetic gangrene or even symptoms of acidosis, reach an age of 60 or 70 years and then die of some other malady. The author has observed several of these baffling cases.

Of the many theories of diabetic glucosuria, two have gained a prominent place in the literature. That of Chauveau and von Noorden claims the trouble to be sugar overproduction in the body; but the more plausible and acceptable one refers the difficulty to imperfect utilization of the carbohydrates

by the tissues. At any rate, hyperglycemia, or sugar-saturation of the blood occurs. Glucose is an excellent diuretic and is excreted mainly or entirely by the kidneys. The intensity of the polyuria varies directly with the amount of glucose excreted by the kidney.

More rarely other forms of glucosuria may be met by the physician. Thus in injuries to the medulla, the output of glucose may be great, and such cases present a very unfavorable prognosis as a rule. The phloridizin glucosurias are explained as a rule by the splitting of this substance into sugar and phloretin. Renal glucosurias are characterized by the fact that hyperglycemia does not occur. Klemperer has ventured the explanation that the renal epithelium becomes morbidly active excreting the physiological sugar of the blood very rapidly, and continues to do so as the deficiency is replaced.

An antagonistic relation between the pancreas and adrenal glands has been supposed to exist with reference to the determination of the glucose content of the blood. It is scarcely necessary to rehearse the well-known fact that extirpation or disease of the pancreatic gland results in glucosuria, and that many if not all cases of diabetes are pancreatic in type. Moreover the injection of substances from the adrenal may cause glucosuria. It has been argued, therefore, that one organ acts physiologically as a check upon the other, and that pathological glucosuria which cannot be attributed to pancreatic disease may be explained by hyper-

activity of the adrenals. This theory is interesting but lacks final proof. However a striking feature in at least two of the atypical cases of diabetes mentioned above, is that both patients suffer from hypertension and albuminuria.

To repeat, the pathological glucosurias *most frequently* met by the practitioner (and which have come to fall under the general heading, diabetes mellitus) must be considered, according to the consensus of opinion, as anabolic shortcomings on the part of the tissue cells toward glucose. In other words, for reasons which we do not clearly understand, the body cells are unable to use the sugar brought to them, and it must pass by them and be excreted while they suffer sugar-starvation. Whether these anabolic shortcomings are due to the absence of some specific ferment formerly supplied by the pancreas or other organ, or due to nervous influences, or both, we are not yet ready to declare.

Glucose, Its Detection.—No one test is without its shortcomings and so several will be described.

Spoon Test.—This is most easily applied at the bedside. Dilute some of the urine with double its volume of water. Place about six drops in a spoon and slowly evaporate to dryness with gentle heat. Then heat again very slowly, when all at once a typical orange-brown color and a characteristic odor of caramel will prove the presence of glucose. Other urines give a smoky black spot and merely a urinous odor. This test is fairly sensitive. It may serve very well when reagents are not at hand (sud-

den coma suspected to be diabetic in origin); but it is well to apply other tests later.

Haines Test.—The Haines test appears to have replaced the Trommer and Fehling tests with the majority of laboratory workers, not only because of its simplicity but because it is satisfactory as well. It must be remembered that the reagent and not the urine is to be boiled. The reagent need not be made up fresh for each test but should be discarded if any precipitate appears in the bottle or should be thrown down in the boiling. It is made up as follows, and attempts to shorten the method of preparation are likely to lead to trouble: Make a perfect solution of 30 grains of pure copper sulphate in one-half ounce of distilled water. Mix thoroughly with a half-ounce of glycerin. Finally add 5 ounces of clear liquor potassæ. The reagent is fairly stable and may be kept in a rubber stoppered bottle almost indefinitely.

Boil 5 c.c. of this reagent in a test tube. Hold in the light to see that no reduction has occurred. Quickly add 5 to 7 drops of the filtered urine, *not more.* Keep the reagent at the boiling point until the moment of adding the urine, and then remove from the flame. *A copious and heavy* yellow, green or brown, sand-like precipitate indicates the presence of glucose. A slight, flaky-white or dirty collection of crystals or flocculent material indicates either a partial reduction of the reagent, albuminous bodies, or phosphates. A delayed action (appearing a minute or so later) is probably due not to

glucose but other reducing substances. The characteristic reaction once seen is rarely mistaken for a pseudotest. It is the latter which is taken for the glucose trace by the unskilled observer.

Fermentation Test.—The Haines test is almost specific for glucose. Clinically in the hands of a man who has applied it many times, it is quite specific. From a strictly scientific standpoint the fermentation test is always specific. In the fermentation test the glucose is broken up into alcohol and carbon dioxid, the evolution of gas being proof of the alteration. Other reducing bodies which might be confused with glucose, will not ferment. The test may be set up in an ordinary bottle or in a special saccharimeter such as will be described in connection with the quantitative uranalyses (see page 101).

The urine should be kept warm during the test, preferably by an incubator, and must be free from preservatives. Any commercial yeast which is glucose-free may be used, but the compressed yeasts are best of all. The only objection to the test is that some time is required for fermentation to take place, but this is not a great objection inasmuch as chronic rather than acute cases demand this work. A very small amount of yeast is sufficient but it should be well mixed with the urine sample by shaking.

Cole Test.—The Cole test for glucose is perhaps the most sensitive of all the clinical glucose methods; and in the question of traces, is considered by

the author as our best rapid method, even more sensitive than the fermentation test. In fact Cole* claims that in devising the perfected technic, the greatest difficulty lay in the fact that it was likely to show the presence of normal amounts of glucose. In a dry, large test-tube, place about one gram of pure blood charcoal. Add 10 c.c. of the urine and shake from side to side to mix thoroughly. Heat to the boiling point, shaking the whole time. Cool thoroughly under the tap and shake thoroughly for about five minutes. Filter through a small paper into a rather wide test tube containing about half a gram of anhydrous sodium carbonate. When the liquid has filtered through, add 6 drops of pure glycerin, shake and heat to boiling. Note the time when boiling commences and maintain active boiling for 50 seconds, shaking from side to side to prevent spurting. Immediately add four drops of a five per cent solution of crystallized copper sulphate. Shake for a moment to mix the solutions, and allow the tube to stand without further heating for one minute. With normal urine, the color remains blue, with a variable amount of a grayish precipitate of the earthy phosphates.

If glucose is present to the extent of .02 per cent or more above the average normal amount, the blue color is discharged and a yellowish precipitate of cuprous hydroxid forms. The rapidity with which the precipitate forms is a measure of the amount of glucose present. With .05 per cent, it appears in a

*Lancet, London, September 20, 1913.

few seconds. With .02 per cent it may not appear until 50 seconds. A reaction taking place after 60 seconds must not be taken as evidence of abnormal glucosuria.

The chief principles involved in this test, are:

1. Charcoal in a certain percentage absorbs the greater part of the nonsaccharine reducing substances of normal urine, the greater part of any lactose which may be present, and so on.

2. Cole claims that certain advantages may be realized by this special mixing of carbonate, glycerin, and copper. This is doubted by the author of this book, who sees in it merely an unnecessary modification of Haines method well known to all American laboratory men. The method may well be termed the Cole modification of Haines test. It may be well in puzzling Haines reactions to treat the urine with charcoal as advised above, cool it and then filter, using the filtrate with the boiling Haines reagent. This appears to be as satisfactory as the methods described by Cole.

Glucose, Sources of Error.—The Haines reagent must be transparent and must not reduce itself when boiled. Excessive amounts of urine must not be added—the limit for 5 c.c. of reagent is seven drops of urine. Do not boil after mixing. The dirty flocculent sediment which sometimes may be observed is a precipitate of earthy phosphates, or possibly albumin if this has not been removed. The glucose sediment should not only be heavy, or sand-like, but is usually copious. Moreover it should ap-

pear rather promptly. A partial reduction appearing after a couple of minutes is likely to prove to be other sugars or nonsaccharine reducing substances. Specimens of urine submitted in triturate vials (lactose), maple syrup containers and patent medicine bottles should be examined with considerable caution. It is well to repeat that the pseudotest is more often mistaken for a trace of glucose than is a typical glucose reaction for a pseudotest. Here as in many other questions, laboratory men will find that "practice makes perfect." The man who is uncertain of his technic should work with controls containing known percentages of glucose.

Uric acid, salicylic acid, hydroquinone, paracatechin, and other substances may cause a reduction especially if the technic is not carefully carried out. However it will be found that the reaction is usually delayed or incomplete. In this connection it is well to keep in mind that dependence is to be placed more upon characteristic precipitation rather than change of color of the reagent. But in case of further question and especially if the specific gravity is not elevated, it is well to resort to the fermentation test or to the Cole test. A trace of serum albumin will not seriously interfere with the Haines test. A large amount of albumin may confuse. In such case, add a drop of acetic acid to 10 c.c. of the urine in case it is alkaline, boil and filter through a clean paper. Use the filtrate as unknown.

When setting up the fermentation test, the specimen must contain no preservative as the action of

the yeast will be inhibited. The reaction will not occur in a cold room, and is most rapid at 25° C. Some commercial yeasts act very slowly, and some are inert. Brewer's yeast or the ordinary compressed yeast kept on ice, is best for use. If the urine appears to contain bubbles of air or other gas, it is best first of all to boil and then permit to cool before setting up the test.

Pentoses, Significance and Detection.—The pentoses (arabinose, xylose, rhamnose, and so on) occur in fruits and vegetables. Urinary pentose is usually an optically inactive arabinose. It has been found in diseases of the pancreas in company with dextrose, but sometimes alone. Cases of essential or crytogenic pentosuria have been met with, where there is no dextrose, no polyuria, no thirst and often no symptoms. The specific gravity of the urine is usually above 1030 and a positive reaction with the reduction tests often leads to a diagnosis of true diabetes (glucosuria). However, the sugar is not broken up by yeast fermentation, and this should place the physician on his guard.

Pentose may be identified positively by Bial's test. One-half gram of orcin in 500 c.c. of 30 per cent hydrochloric acid, and 20 drops of liquor ferri sesquichloridi are added. This will serve as reagent and is stable for several months. About 5 c.c. or somewhat less of the reagent are heated in a test tube until the boiling point is reached. Do not, however, permit it to boil. As soon as the tube is removed from the flame, 10 drops of the urine are

added. Within a few seconds, the liquid turns green if pentose is present. A green tint occurring a minute or so later may be due to glycuronic acid. If glucose is present, it will give the orcin test and should first be removed by fermentation. In highly colored urines (concentrated urines where normal reducing substances are increased per c.c.), animal charcoal should first be added, and after heating, the mixture may be filtered and the clear filtrate may be tested. However, it is well to remember that pentoses occur in some filter papers and may be dissolved out especially if the urine is highly acid. In case of question, run controls alongside.

Where glucose is not present and it appears that we are dealing with a single sugar, positive identification may ofttimes be made by Neumann's modification of the orcin test. It is necessary here for the safety of the worker that a wide test tube be used and that the mouth be directed away as the reaction is sometimes attended by explosive violence. Five or seven drops (not more) of the urine are placed in the tube and then are added 5 c.c. of glacial acetic acid and 5 drops of a 5 per cent alcoholic solution of the orcin. Finally we heat the mixture to a boil. Then add sulphuric acid carefully, drop by drop, holding the mouth of the tube away from the face to guard against explosion. After adding each five drops, shake the tube. When a first permanent color is secured, add no more acid. It may be necessary to add quite a little acid but never more than 50 drops are necessary. Do not boil the

mixture at any time. When the temperature has reached the boiling point, remove from flame and then add the acid. Finally cool. By this method it is easy to discriminate between the sugars, if but one is present. The pentoses give violet hues; glucose, brown, and glycuronic acid, green. The latter substance though an aldehyde is closely related to the sugars and is responsible for many of the pseudotests for them.

Lactose, Significance and Detection.—At some points in lactation, tests of the mother's urine are quite likely to show the presence of sugars. The reaction to Haines test may be delayed but is positive for lactose. Lactose is most likely to be found in the urine of the mother when the breasts are distended with milk. Distinction rests not alone upon the clinical circumstances but upon the fermentation test which breaks up glucose but does not affect lactose.

Diacetic Acid, Significance.—The acetone bodies (acetone, diacetic acid, beta-oxybutyric acid, and other substances) play an important if not the chief part in the acidoses of diabetes as well as in other toxemias as yet not perfectly understood. When all evidence is in, we are not certain as to the identity of the acetone precursors. It is fairly evident that they do not arise from the sugars but represent an attempt either upon the part of the proteids or the fats to split into products of two types, one the needed carbohydrate for the cell, the other the waste products resulting from such cleavage. At

any rate it is probable that the acetones are indicative of "carbohydrate starvation" where as in diabetes, the tissues are unable to use the carbohydrates brought to them and past them, or where as in postanesthetic vomiting, appendicitis, pregnancy and so on, the tissues are clamorous for sugars but either these have not been supplied (diet before anesthetic usually is meager), or if supplied have not been assimilated by the portal circulation (appendicitis). In consequence, proteids or fats are broken up to gain their carbohydrate portion, and poisonous remnants are left.

Coincident to the appearance of acidosis, falls the urea. This must not be mistaken for retention but rather as failure of elaboration. For when the acids and acid-like bodies are freed into the blood and lymph, the ammonia combines in part to neutralize them, that the fixed alkalies of the tissues may be spared. The ultimate antecedent or at least the representative of such is supposed to be beta-amidobutyric acid; but it has not been identified in the urine.

Diacetic Acid (Aceto-acetic Acid), Detection.—Routinely we rarely test for other of this series than diacetic acid. These bodies are very volatile so that tests should be made as soon as possible. In the Gerhardt's test the urine must be added to reagent, instead of reagent to urine as is usually advised.

Gerhardt's Test.—To about 10 c.c. of a clear, strong solution of ferric chlorid, add 2-3 drops of the urine. A wine-red color indicates the presence

of diacetic acid. A deep red, purple, or brown color indicates the presence of salicylates, coal-tar products and so on. This is best demonstrated by the fact that a control set up with some of the urine which has been boiled will show a color quite as intense whereas if it were diacetic acid, this being volatile, no or but little color would appear. As a matter of fact these drugs should not be exhibited before the test is set up, as they will obscure the true diacetic acid tint and the examiner be left in question.

Lindemann's Test.—Shake together 10 c.c. of the urine, 5 drops of acetic acid, 5 drops of Lugol's iodin solution (or double amount of this solution if urine is charged with uric acid), and 3 c.c. of chloroform. With urine containing diacetic acid, the chloroform is not colored. With other urines a pink or red chloroform results from the escape of unbound iodin. This specific binding is accomplished by diacetic acid.

Acetone, Detection.—We have considered above the meaning of acetone in the urine. We usually test for diacetic acid and in case of further question seek to detect acetone. This occurs in merest traces in the normal urine, but is not shown by the Trommer test unless it occurs in abnormal amounts. It may be present either in the urine or the breath in amounts sufficient to be detected by its odor. It has been ventured that more acetone leaves the body by the expired air than by the urine. This odor has been variously described. It approaches that of ap-

ples just beginning to rot. It is characteristic and once perceived is rarely mistaken in the future.

Trommer Test.—The Trommer test has become very popular in many laboratories; and for routine purposes is much to be preferred to the older methods. It is a very sensitive test. Add a gram of dry potassium hydroxid to 10 c.c. of the urine; and to this alkalinized urine in turn add 10-15 drops of salicylic aldehyde. Warm the mixture in a flame but do not raise to boiling (rather to the temperature that it just burns the palm of the hand). A red or purple ring at the line of contact proves that acetone is present.

The Indicans, Significance.—So nearly as we are able to judge, indicanuria invariably signifies the occurrence of proteid decomposition at some point in the body. Certain authorities have ventured to gainsay this conclusion, possibly because the focus of putrefaction is not always evident. However, the recent studies of the etiology of toxic (infectious) arthritis and certain forms of nephritis, show that hidden and symptomless infections are fairly common. It follows that many of these indicanurias which cannot be traced to the bowel may be thus explained. However, in the great majority of cases morbid biological processes in the colon must be held to account.

Indicanuria is not to be regarded as an index to simple constipation. It may result from proteid gluttony (the American dietetic sin). It may result from incomplete digestion, absorption and assimila-

tion of the proteids higher in the alimentary tract. (After all, there is but one bowel; and anatomical divisions are sometimes misleading.) In enteroptosis the formation and absorption of indicans is enhanced.

What occurs is this. Because of colonic stasis, or for other reasons, certain bacteria act upon the food-proteids, breaking them up into a large number of more or less poisonous bodies. The bowel serves merely as the incubator. These bodies are taken up in part by the blood and eventually reach the kidney. The symptomatology has been debated, but symptoms undoubtedly occur and are variable. However, they are well known to most physicians and need not to be detailed here. A hairsplitting debate has rendered the term, autointoxication undesirable. We now speak of the condition as intestinal toxemia (copremia), another term which has been criticized, but which will answer well enough until these critics have devised a better one.

We have spoken of the role of the acids (see page 38). Indican proper is the product resulting from the oxidation of indol and its subsequent conjugation with simple sulphates.

The Indicans, Detection.—Mix in a test tube, 5 c.c. each of urine and hydrochloric acid. Add about 2 c.c. of chloroform and a couple of drops of reliable peroxid of hydrogen. Place the thumb over the open end of the tube and gently shake or invert several times. If a reaction fails, shake again or add a few drops of alcohol and shake.

The ordinary indican is blue indican. If it is present in traces it will impart to the chloroform a delicate sky-blue color. In larger amounts the chloroform will become as dark blue as a five-cent postage stamp. Excessive quantities of indican give a violet hue while in severe tuberculous enteritis, strangulation and so on, the chloroform may be quite black. Usually the entire contents of the tube may show some color although most of it is concentrated in the chloroform.

Indigo red or urorosein gives a pink or red color to the chloroform in exceptional substances. Any urorosein which may be present is usually obscured by the blue indican. Urorosein is supposed to be a skatol derivative.

The Indicans, Sources of Error.—In routine laboratory work the chief pseudotest may be explained by the iodids. The chloroform becomes pink, and the color is quickly discharged by setting in direct sunlight or by reshaking after the addition of alcohol. Urorosein should always be reported with reluctance. It is rarely found and inquiry will usually reveal the fact that the patient is taking iodids. Moreover proof may be had by Lesser's test. He stirs with the end of a match stick a little calomel into a few drops of the urine on a slide. If it contains iodin, the calomel becomes bright yellow. In case of further question run a control alongside with normal urine. In all cases of importance, do not administer salicylates, urotropin and iodids before testing for indican.

Bilirubin may give a pseudotest, but it may be identified by other tests and the error shown. When chloroform has been added to a sample to preserve it until it can be examined, it is well to test this for indican as the chloroform rather than the urine may hold the indican in solution.

Too much of the peroxid must not be added or else the blue color will be discharged as quickly as formed. A trace of indican should never be reported in a urine containing considerable albumin, nor should a trace of albumin be reported in a urine containing tremendous quantities of indican. Indican has no significance as a rule in purulent infections of the genitourinary tract for we have a local focus of proteid decomposition.

Indolacetic Acid.—This is one of the acids formed in proteid decomposition and may be present either with or without indican. To 5 c.c. of the urine, add a drop of one per cent potassium nitrate and a few drops of hydrochloric acid. A pink color shows the presence of indolacetic acid. Its significance is identical with that of indican.

Urochromogen, Significance and Detection.—Although a precursor of other pigments, urochromogen does not appear in the normal urine. Weiss and others consider the urochromogen reaction to be a part of the diazo test and even more sensitive than that devised by Ehrlich. Urochromogenuria occurs in late pulmonary tuberculosis, and is of prognostic rather than diagnostic value. It seems to argue that the case is hopeless; and it is useless to administer

tuberculins any longer. With clear, fresh urine fill a test tube one-third. Fill the remainder of the tube with distilled water. Place half of the resulting mixture in another tube to serve as a control. Add to one of the tubes, three drops of a 1-1000 solution of potassium permanganate; mix thoroughly and compare with control. A yellow color is regarded as a positive test.

Diazo Reaction, Significance.—The diazo reaction is of great value in the early diagnosis of typhoid fever. It is not absolutely specific for typhoid but is almost so; and fortunately appears very early in the course of the disease. The technic is easily and rapidly completed. The reaction is almost always present, and occurs less frequently in diseases likely to be mistaken for typhoid. It appears in miliary tuberculosis, but usually somewhat late in the course of the latter disease. This reaction should be carried out alongside the Russo and the Gruber-Widal agglutination test, there being between the three an interdependence very valuable to the diagnostician. This may be expressed in a table* (see next page). Recently the author has found that the diazo is sometimes present in the paratyphoid infections, whereas, as is well known, suspensions of the paratyphoid strains must be used in order to secure a positive agglutination reaction. Typhoid vaccination may lead to a positive Widal in a case which is not typhoid, but has no effect upon the diazo test.

*Williams, B. G. R.: Archives of Diagnosis, January, 1912.

	Widal	Diazo	Russo
Appears When?	Appears in typhoid fever. Usually after first week.	Appears early in typhoid fever.	Appears in typhoid fever.
Relapses and complications?	Relapses or complications appear to have no effect on reaction.	Reappears with relapses but not with complications.	A positive reaction which grows in intensity probably points to a grave or fatal issue.
Is the technic difficult?	Several methods: some difficult, others fairly simple.	Technic simple.	Technic exceedingly simple.
Reaction time?	Observations of 2 to 24 hours necessary.	Technic rapidly completed.	Technic rapidly completed.
Acute miliary tuberculosis?	Never present in this disease.	May appear in this disease.	Perhaps never present.
Always present in typhoid?	No.	No.	Yes, perhaps in every case of true typhoid.
In measles?	No.	Sometimes.	No.
In pneumonia?	No.	Sometimes.	?
In malaria?	No.	Sometimes.	No.
In small-pox?	No.	No.	Yes.
Chronic tuberculosis?	No.	Yes. Often occurs late in fatal cases.	Yes.

Effect of drugs taken internally?	None.	It is probable that no drug taken internally gives the true diazo reaction, although the differentiation of some of these pseudo-tests may require some skill. Certain of these drugs may interfere with the positive test.	The positive test seems to be given by several drugs, notably urotropin. Also by bilirubin in jaundice.
Reaction late in typhoid?	The rule.	A diazo test which makes its first appearance after the second week points to acute miliary tuberculosis. When a positive reaction suddenly appears during convalescence a relapse is to be expected.	?
Persistent reaction in typhoid?	Gives no diagnostic nor prognostic information.	May persist througnout infection without any special significance.	Persistent reaction probably spells a bad prognosis.
Significance of negative test.	None.	None.	A negative test when persistent in an established fever, speaks against typhoid.

Diazo Reaction, Technic.—The following reagents are used:

Diazo Reagent No. 1.—Sulphanilic acid; saturated solution in 5 per cent hydrochloric acid.

Diazo Reagent No. 2.—Sodium nitrite; 1/2 per cent aqueous solution.

Diazo Reagent No. 3.—Aqueous ammonia.

By the aid of a pipet place into the test tube 51 drops of the specimen, 50 drops of solution 1 and a single drop of solution 2. Mix thoroughly but avoid the formation of a foam if possible. Then quickly add half a dozen drops of pure ammonia, permitting it to flow down the inner side of the tube and out upon the surface of the mixture in such a way as to form an upper stratum. In case the diazo is positive, a beautiful pink or rose-color will be seen at the junction. Shake thoroughly; the foam should likewise be red or pink. Later a dark green sediment may form in the liquid. Pseudotests are given by many medicaments, but such reactions are rarely pink or rose-red but are yellow. However inquiry should be made into the taking of opium, salol, antipyrin, and so on.

The Russo (Rosso) Reaction.—The Russo reaction appears in many fevers. It is of chief value, perhaps, when absent because a negative test speaks very forcibly against the diagnosis of typhoid fever. The author has seen this point brought out many times. A typhoid state (with or without fever) in which the Russo is absent, is very likely to turn out to be a nephritis, brain tumor, meningitis, or condi-

tion other than typhoid fever. Moreover the Russo is rarely or never present in miliary tuberculosis, and its value here is secondary only to the above.

The Russo reagent is a 1-1000 aqueous solution of methylene blue. Four drops are added to 5 c.c. of the urine. A positive reaction is shown by the presence of a deep emerald or mint green color. A bluish or greenish tinge is regarded as a negative reaction.

Chemical Identification of Sediments.—Pus is easily identified by the addition of a solution of caustic soda which causes the proteids to gel and become tenacious (so-called muco-pus).

The following table (Heller) outlines the proper procedure for the examination of calculi:

Heat some of the dry material on a platinum foil.

1. It does not burn. Treat it with hydrochloric acid.
   - A. It does not effervesce. Treat it with hydrochloric acid and heat gently.
     - I. It does not effervesce. Moisten some of the powder with a little potassium hydrate.
       - a. Ammonia freed. *Triple phosphates.*
       - b. Ammonia not released. *Earthy phosphates.*
     - II. It does effervesce. *Calcium oxalate.*
   - B. It does effervesce. *Calcium carbonate.*

2. It does burn.
   - A. With a flame.
     - I. Yellow flame with an odor as the burning of flesh. *Fibrin.*
     - II. Blue flame and soon ceases. Sharp odor. The original powder is soluble in ammonia. Permit some of the solution to evaporate and observe characteristic hexagonal plates. *Cystin.*
   - B. Without a flame.
     - I. Does not give murexid test. *Xanthin.*
     - II. Gives murexid test. Treat some of the powder with sodium hydroxid.
       - a. Ammonia is loosed. *Ammonium urate.*
       - b. No ammonia freed. *Uric acid.*

CHAPTER IV.

QUANTITATIVE URANALYSES.

Only those quantitative uranalyses of diagnostic value have been detailed in this chapter. Certain other estimations are sometimes carried out but they give no usable clinical evidence, and for these the worker is referred to books on physiological chemistry.

Total Solids.—Practically all methods give approximate results only, but approximations suffice for clinical work. By evaporating the urine and weighing the residue (a laborious procedure) the estimation may be made, but this does not guarantee absolute accuracy inasmuch as ammonia is driven off.

The method which is most satisfactory for clinical purposes is as follows: Multiply the last two figures of the specific gravity by the number of fluid ounces excretion in the twenty-four hours and the result in turn by 1.1, which gives the total solids in grains. This figure divided by 15, gives the reading in grams. Normally the solids each twenty-four hours, vary between 40-80 grams, but 64 grams is the average in urines of perfectly healthy individuals. The following example will make this method clear. Suppose the patient passes 45 fluid ounces of urine

daily, and the specific gravity of the mixed specimen is 1019.

$$(45 \times 19 \times 1.1) \div 15 = 62.7 \text{ grams.}$$

Normal Quantitative Composition.—The following table expresses approximately in terms of grams, the solid composition of the urine each twenty-four hours:

Urea	35.
Chlorids	12.
Acid phosphoric	5.
Acid sulphuric	3.
Earthy phosphates	1.5
Acid uric	1.5
Creatinin	1.5
Acid hippuric	1.5
Ammonia, iron, pigments, etc.	3.
Total	64.

Keep in mind, however, that these figures are approximate even in health, since diet and other factors subject them to great variations. In a bedfast person the total solids are greatly decreased and it is not unusual to find but 20 grams of urea in such a person even though there is no renal lesion.

Chlorids.—Man excretes from 10 to 14 grams of common salt by his kidney each twenty-four hours. This amount is lowered by a reduction of certain foods which contain salt, and is increased vice versa. Regardless of the amount taken as food, the chlorids are decreased in acute fevers and in certain forms of nephritis. In pneumonia, the urine may become practically salt-free, but tremendous quan-

tities are again passed just preceding the crisis. In suspected nephritis where the food is rich in salt, a low excretion is presumptive of kidney injury (retention of chlorids). This contention has been disputed upon theoretical grounds, but observation in a large number of cases will convince that "chlorid retention" may occur before albuminuria and cylindruria and many months before urea retention can be proved. In such cases, foods containing salt should be reduced; and salt should not be used for seasoning inasmuch as the retention of sodium chlorid in the tissues favors edema and other serious complications.

Coarse Method.—This answers very well in many cases. Remove albumin by heat and filtration when necessary. Filter in case the urine is not transparent. To 10 c.c. of the urine, add four drops of 10 per cent silver nitrate solution. Thick, curdy precipitate indicates normal chlorids while a mere milkiness is proof of diminution. Use control of a normal urine in this test.

Titration Method.—The following reagents are prepared:

Reagent No. 1.—Dissolve 29.06 grams of pure silver nitrate in one liter of distilled water. One c.c. of this solution corresponds to .01 gram of sodium chlorid (or each c.c. corresponds to 10 milligrams of sodium chlorid).

Reagent No. 2.—A saturated, chlorin-free solution of potassium chromate (yellow). This serves as indicator.

Method.—Remove albumin if necessary. Place in each of two large evaporating dishes, exactly 10 c.c. of the urine, two drops of indicator and enough distilled water to make up 100 c.c. To dish one, we add drop by drop, from a buret the Reagent I, until in spite of stirring with a glass rod, a faint red or pink color becomes perceptible. This may be recognized more quickly by contrast with the urine in the control dish. The buret reading is then taken. (A drop or two of the silver will make permanent the red color and show that the above reading was correct.) Having determined the sodium chlorid present in 10 c.c. of the urine, it is easy to calculate the amount present in the twenty-four hour sample.

For example:

Amount of urine per twenty-four hours=1200 c.c.
Amount of reagent used to the 10 c.c. of urine=12 c.c.
Amount of sodium chlorid present in 10 c.c. of urine=.12G.
Sodium chlorid present in twenty-four hour sample=14.4 G.

Phosphates.—Of much less practical importance is the determination of total phosphates. The clinical significance of the so-called "phosphaturias" is not clear. Inasmuch as reliable quantitative methods are complicated and afford no diagnostic data, it has seemed best to omit them from this volume. A few words are pertinent. To phosphates (acid or alkaline salts) is due the normal urinary reaction. Certain cases of "phosphatic diabetes" have been studied. Clinical phosphaturia (earthy phosphates in large amounts with a fixed alkalinity of the

urine) occurs in certain functional nervous diseases. Von Noorden has shown that phosphates even in normal amounts irritates diseased kidney parenchyma, but this holds perhaps in the case of most of the inorganic urinary salts.

Sulphates.—For practical purposes it is not necessary to titrate for total sulphates. Where ethereal increase is sufficient to demand clinical considerations, the indicans are present and may be detected by simple color tests.

Total Nitrogen.—In routine work, the estimation of the total nitrogen is not necessary. Because of the fact that a certain quantity of the nitrogen of the food and perhaps also of tissue metabolism, escapes by the bowel, the total nitrogen of the urine cannot be regarded as an absolute index of proteid metabolism. Inasmuch as urea bears an almost constant ratio to the total nitrogen and inasmuch as it is eliminated almost entirely by the urine when the kidneys are not seriously diseased, urea estimations are of far greater importance.

The waste nitrogen of the food and of tissue metabolism is excreted as urea, ammonia, uric acid, creatinin and amino-acids (the latter with ammonia, especially where there is a disturbed formation of urea as in hepatic disease).

Ammonia and Amino-acids.—Urea is the non-poisonous synthetic resulting when carbon dioxid and ammonia (two poisonous, waste-products of katabolism) are united in the liver. In certain hepatic diseases, this elaboration is effected only in

part or not at all, and in consequence much of the urinary nitrogen occurs as ammonia and amino-acids rather than urea. Then, too, in acidosis and certain acidemias the ammonia is used to neutralize the poisonous acids that the fixed alkalies of the tissues may be spared. Being, therefore unavailable for the synthesis in the liver, the urea is decreased in diabetes and similar affections.

Ammonia occurs in the normal urine, about .9 grams being excreted daily. Various methods for estimating the urinary ammonia, have been suggested. Any of those approaching simplicity are inaccurate. As a matter of fact we know that in cases where ammonia estimations are likely to prove of value, the urea is considerably lowered, and this is evidence presumptive, providing we are able to rule out urea retention of kidney lesion. Moreover titration shows excessive acidity in many of these cases. As a rule it is best to leave ammonia studies to the research worker and large laboratories, and give chief attention to urea.

Ammonia Estimation.—A fairly accurate method is that of Schlösing, though the readings are usually a bit too low. The urine should be fresh, or if sent to a laboratory, five drops of 5 per cent potassium fluoride should be added to prevent the breaking up of urea into ammonia. The mouth of a bell jar is ground and fitted to a ground glass plate. The edge is smeared with tallow to prevent the entrance of air. A Petri dish containing 10 c.c. of decinormal sulphuric acid is placed on the center of the plate.

On this in turn is placed a triangle bearing a beaker containing 20 c.c. of the urine. To this urine is added 20 c.c. of milk of lime. The bell jar is placed over the whole to make the chamber air-tight. After forty-eight hours the acid is titrated and thus the amount of ammonia absorbed is ascertained.

Urea.—Having thus placed chief stress upon the value of the urea estimation, it is very necessary that this be made as accurately as possible. Properly mixed and preserved twenty-four hour specimens should be used, as the urea excretion varies from hour to hour and percentage readings from single specimens are likely to mislead.

The methods which in the hands of the author have given the best results are those of Doremus and of Squibb. With the latter apparatus, details and charts are supplied, so that a description is not necessary here.

The Doremus estimation is made as follows: Make up a solution of sodium hydroxid, 100 grams to 250 c.c. of distilled water. This is a stock solution and must be closed with a rubber stopper. When ready for the test, pour into the Doremus ureometer enough of this hydroxid solution to fill the tube to the mark "═." Now by means of the pipet which accompanies the instrument, add one c.c. of pure bromin to the hydroxid solution. When this has been dissolved, dilute by pouring in distilled water until the long arm and the bend of the ureometer are filled. When the proper amount is added, the liquid should be thoroughly luted at the bend of the

arm and no air remain in the long arm when the instrument is upright. By properly tilting the instrument during this manipulation, this may be done. Take 1 c.c. of the urine in the clean, curved pipet, immerse the tip under the bend in the tube

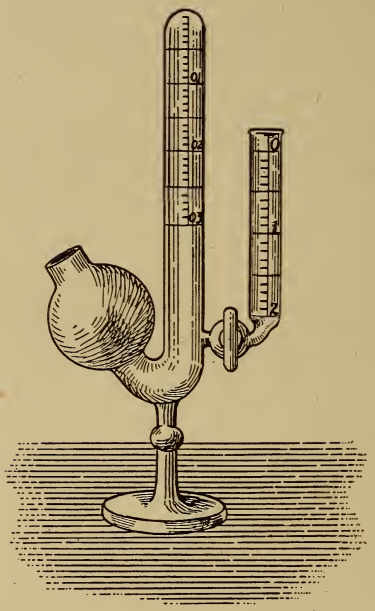


Fig. 3.—Doremus-Hinds ureometer.

and discharge all of this urine into the hypobromite, being careful that air does not enter either from the air behind the urine column or from the outside of the tube by improperly tilting. Permit the apparatus to stand thirty minutes. Then read amount of solution displaced (in terms of grams).

It is easy to calculate for the twenty-four hours. For example suppose that the reading for 1 c.c. is .023 grams. The twenty-four hour quantity of urea is 1500 c.c. By simple multiplication the total quantity of urea is 34.5 grams. Highly concentrated urines may need to be diluted with known amounts of distilled water, to be used with this instrument. When buying a Doremus ureometer, request the French system of marking if the above directions are to be followed. The English ureometer gives the readings in grains.

The twenty-four hours' urea estimation is the best laboratory procedure to determine the functional activity and possibilities of the kidneys; i. e., just how much work they are doing. Special methods must be used in surgical kidney where the function of the single organ is in question, or it is necessary to determine which of the two is diseased.

Uric Acid.—Anent the so-called "diatheses," it may be said that in gout and certain other disorders, uric acid has borne the blame for many years. There can be no question but that uric acid deposition plays a part in true gout; but this part is, perhaps, secondary. But the various forms of arthritis and the like, formerly attributed to uric acid "diathesis," have been seized upon by the pathologist and properly placed under other heads, notably under the infectious toxemias. So far as the uranalysis is concerned in gout, especially in acute gout, the uric acid excretion is reduced, except at periods when it is excessive. To be sure, the uric acid in the

blood is high, but by no means higher than in other chronic diseases or indeed than after a heavy proteid meal. It may be advisable to keep tab on the uric acid excretion in gout (especially as regards certain diets or drug treatments) and for this reason the following method for estimation may be detailed:

Ruhemann's uricometer is recommended for routine clinical work. The reagent consists of iodin 1.5 grams, potassium iodid 1.5 grams, and 15 grams of absolute alcohol in 185 c.c. of distilled water. The test is set up by filling the instrument to the first line "S" with carbon bisulphid. Then by means of a pipet, Ruhemann's reagent is added until it stands at the next mark "I." On this is allowed to run the urine (which should be at a temperature of 65 degrees F.) until it stands at the lower part of the graduated scale marked 2.45. This is best accomplished by means of a pipet. The open end of the instrument is then closed by the stopper and the tube shaken. The carbon bisulphid becomes brown. Remove the stopper and add more urine. Shake and observe the bisulphid. Cautiously repeat the process until the bisulphid becomes white. Then read the upper level of the liquid which shows in grams the amount of uric acid per 1000 c.c. of urine. Alkaline urines must be acidified slightly with acetic acid before the test is set up. Likewise if albumin is present, it must first be removed by heat and filtration. Where there is very little uric acid, it may be imperative to add the iodin reagent but

to the line midway between "S" and "I" and divide the reading by half.

Nowadays the physician will have but little use for the uric acid reading, since the clinical significance of the estimation has been shorn of its former glory.

Oxalic Acid.—Clinical oxaluria, that is an oxaluria causing local symptoms (oxaluria dolorosa), is diagnosticated by finding the minute crystals of calcium oxalate in the freshly voided urine. True oxaluria is detected by quantitative estimation of the oxalic acid of the urine. Inasmuch as it has been shown that either condition may exist without the other, the quantitative estimation of oxalic acid is not called for in routine diagnostic work.

Indican.—Indican is a strictly abnormal urinary finding, and the success of a treatment is best shown by the absolute disappearance of indican from the urine. Color charts are of some value in determining indican decrease, but offer no advantage over the scheme of watching the chloroform becoming lighter blue in color and finally colorless when indican is absent.

Diacetic Acid.—The same applies to diacetic acid. There may be a profound diabetic coma with but little diacetic acid in the urine, and a trace is distinctly pathological. These tests are usually classified as to degree by the intensity of the color as follows: overplus, decided, moderate, small amount,

and none. For practical purposes, this nomenclature is sufficient.

Serum Albumin.—Here as elsewhere the test of a single voiding is valueless. The amount of albumin may vary from hour to hour. Moreover a urine may show a lower *per cent* than at a previous examination because more urine is being passed. Quacks have made use of the percentage test in connection with the administration of diuretics to demonstrate the efficiency of the latter; but a series of twenty-four hour computations will show whether or not albumin has actually decreased. Finally we are not certain that decrease of albumin is a favorable prognostic item, inasmuch as albuminuria may depend not alone upon the nephritis but upon circulatory changes and so on, and albumin may decrease in amount just before the fatal issue, although an increase is the rule.

Fig. 4.—Esbach albuminometer.

Roughly, albumin coagula may be classified as putty-like masses, distinct coagula or flakes, milkoid liquid and mere fogginess.

The volumetric calculation of serum albumin is usually made by Esbach's method. These albuminometers are easily secured from all jobbers. The re-

agent is made up by dissolving in one liter of distilled water, 10 grams of picric acid and 20 grams of citric acid. The test is set up by filling the tube to the mark "U" with some of the mixed twenty-four hour urine specimen (filtered if not transparent) and adding the reagent to the mark "R." The tube is corked with a rubber stopper and the contents thoroughly mixed by inverting several times. It is then set aside for twenty-four hours in a perfectly vertical position. The reading is made at the highest point reached by the sediment. This shows the number of grams of serum albumin to each 1000 c.c. of the urine. For the twenty-four hour specimen the calculation may be easily made by substituting in the following equation:

x=grams of albumin per twenty-four hours.
a=quantity urine passed in twenty-four hours.
b=reading on albuminometer.
1000:a::b:x.

For example:
a=1500 c.c.
b=4.

Substituting:
1000:1500::4:x.
x=6 grams.

A rapid method has been proposed for the Esbach test whereby readings may be made in a few minutes. 10 drops of a ten per cent ferric chlorid solution are added to the urine after it has been placed in the Esbach tube to the mark "U." Then the Esbach reagent is added to the mark "R." The tube is stoppered and the contents mixed as before. Finally place the tube *perfectly upright* in a water

bath at 70° C. Coagulation will be complete in 15 minutes as a rule. The results are accurate.

Glucose.—The chemical methods for the exact estimation of glucose give variable results in the hands of the average worker. This is due to the fact that considerable practice and pains are necessary to recognize the exact point of total reduction.

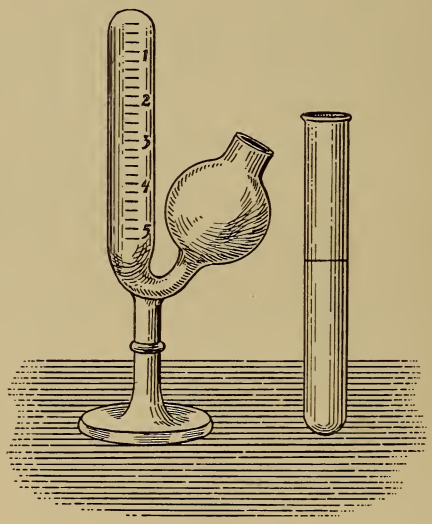


Fig. 5.—Einhorn saccharometer.

Although the fermentation method takes longer for its completion, it is easily done and results are quite accurate if directions are followed. On the Einhorn saccharometer (saccharimeter) the graduations are such that the amount of carbon dioxid loosed may be read off as per cent of glucose. If there is more than a trace of glucose in the urine (as shown by in-

creased specific gravity and preliminary tests), the urine should be diluted 10 times with distilled water, multiplying the reading of course by 10 to get the proper final result.

Fermentation Test.—Acidify the urine if necessary. Shake a small amount of the urine with approximately 10 grains of fresh compressed yeast. Pour the mixture into the saccharometer in such a way that no air is left in the long arm. Place in an incubator or at incubator heat for twenty-four hours or until no more gas bubbles are given off (twelve hours often suffice). Not only will a lower temperature retard fermentation, but it must be recalled that the volume of a gas varies with the temperature. (In a cold room the readings will be too low.) It is best to set up controls with normal urine and urine to which glucose has been added, which will show whether or not the yeast ferments itself (contains glucose), and to what degree; and whether or not it is really active.

The following precautions must be kept in mind:

1. Urine must be slightly acidified unless already acid.

2. Must be properly diluted if there is more than a trace of glucose.

3. Do not shake *too well* the yeast and urine. Although mixture should be fairly complete, continued shaking will include many air bubbles. If the yeast settles before fermentation begins, mix gently.

4. Keep at or near 37° C. for the reasons given above.

5. Use the controls mentioned above to test yeast activity and glucose presence in yeast.

6. Anent urinary antiseptics, it has been claimed that after the administration of drugs of the aromatic series, heavy metals, hexamethylenamin and even some of the alkaloids, fermentation may be thwarted.

7. Do not be misled by the figures etched on the right hand of the scale: these refer to the capacity of the tube in cubic centimeters. Readings are made from the figures on the left.

8. Finally do not forget to multiply by the proper figure if the urine has been diluted.

Haines' Test.—This is the best of the chemical quantitative methods, but it is suggested that considerable experience is necessary in the correct application for the reasons given above. For this calculation, a special Haines reagent is made up as follows:

Copper sulphate, C. P.....	8.314 grams.
Potassium hydroxid, C. P...	25.000 grams.
Ammonia (pure)	350.000 c.c.
Glycerine	40.000 c.c.
Aqua destill.	1000.000 c.c.

Into an Erlenmeyer flask, measure 10 c.c. of this solution and add 70 c.c. of distilled water. Boil the mixture steadily, adding carefully from a buret the urine drop by drop, until the color begins to fade to a light blue. Now add each drop very carefully, shaking the contents somewhat after each drop is

added and waiting a few seconds to see if the color is entirely discharged. When finally the urine is quite colorless, the end reaction has been reached and the reading may be made from the buret. 10 c.c. of the Haines solution are decolorized by .01 gram of glucose.

Suppose for example 3.5 c.c. of the urine were required to decolorize. Then 3.5 c.c. of the urine contains .0028 grams of glucose. Multiplying by 100 will give the glucose percentage, which in this case would be .28 per cent.

CHAPTER V.

MICROSCOPIC URANALYSES

Upon standing, certain of the urinary constituents are deposited. Sometimes this takes place rapidly, at other times after several hours. Many urines contain constituents which have never been in solution (cell, casts, and so on) while in the case of others, the deposition may have occurred in the upper passages (oxalates) or in the bladder (amorphous urates). Moreover it must be kept in mind that the specimen may contain matter from the genital organs, alimentary tract or skin. Certain extraneous substances as dirt, cotton fibers, hairs, starch grains and so on may find their way into the specimen.

The Nubecula.—Soon after a urine has been passed, it may be noted that it is not perfectly transparent. The cloudy deposit, or nubecula is made up of mucus and a few cells from the urinary passages. In this mucus reticulum, specific pathological finds may be suspended. Now and then it may happen that the cloud rises to the surface, bearing in its interstices these important elements. But usually the "urinary sediment" sinks to the bottom of the specimen or may be thrown down by centrifugalization.

Centrifugalization.—Centrifugalization may lead to the morphological alteration of certain of the urinary constituents, but centrifugalization is usually preferred to sedimentation for the following reasons:

1. Urines should be examined fresh because some urines contain pepsin which dissolve casts and other organic constituents.

2. Because of changes due to fermentation. The alterations incident to ammoniacal decomposition render microscopy unsatisfactory.

Fig. 6.—Sedimentation glass.

3. Urines upon standing undergo certain changes even though there is no fermentation. Thus certain salts are thrown down—urates, oxalates, and so on.

4. Some sediments are not sediments in fact, but remain suspended in the urine or actually rise to the surface and may be secured only by centrifugalization.

In some cases the use of preservatives with sedimentation may be used to control questionable finds with centrifugalization. For preservatives see page 20. Delepine has recommended refrigeration to prevent the decomposition of urine, but it must be

remembered that decreasing the temperature of a urine favors the deposition of certain sediments notably the amorphous urates which tend to obscure more important finds as the occasional cast or renal cell.

Practical Microscopy.—A small amount of the sediment is removed from the bottom of the sedimentation glass or centrifuge tube as follows. A

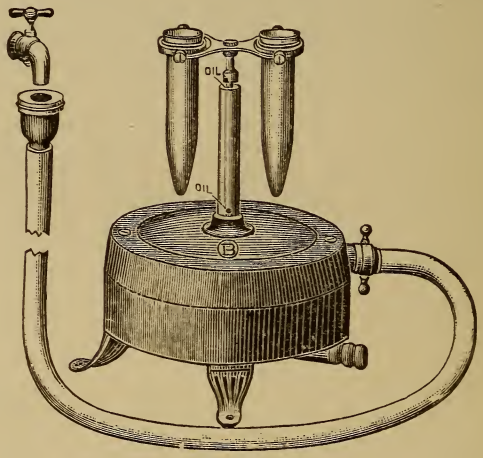


Fig. 7.—Water centrifuge.

pipet is pushed into the sediment, the larger end being closed tightly by the finger. Slightly release the pressure of the finger permitting a small amount of the sediment to enter the pipet, and then close tightly again and remove the pipet. A drop of the sediment may be secured by releasing the pressure of the finger. This may be dropped on a slide and a cover-glass placed upon it for examination. The

point of the pipet should not come into contact with the slide, for more or less than a drop of the sediment will be obtained. The *drop should drop* or fall upon the glass. If too much sediment is secured the cover-glass cannot conceal it and the lighter sediment, as casts, will be forced beyond the edge of the cover, and hence missed in the examination. Or if large drops must be examined, very large cover-glasses may be used.

The ordinary glass slide does not answer the purpose so well in routine work as a piece of window glass cut to the dimensions of 2 inches by 4 inches.

Many important sediments are missed because the microscopist uses too much light. After securing the illumination and the proper focus, it is well to narrow the iris diaphragm until the best view of the sediment is secured. A twilight illumination is best for most sediments.

Some sediments are examined with difficulty. Thus ammoniacal fermentation may not only result in the disintegration of cells and casts, but the latter may be obscured by the mineral sediments thrown down in large quantity. Excessive amounts of blood or pus may hide a few casts or renal cells, and it is sometimes best to make examinations when the urine is comparatively free from blood or pus.

CHEMICAL SEDIMENTS

Sediments are usually classified as mineral and organic. Unorganized and organized, is the nomen-

clature preferred by some authors, and is to be preferred because oxalates, urates and so on (truly organic compounds) must be placed with the mineral sediments to differentiate from cells, casts, and so on. The terms, chemical and biological sediments have their shortcomings, but are perhaps best of all.

Uric Acid.—Of chemical urinary sediments, the form of the uric acid crystal varies most of all. The size and color also varies. The barrel or whetstone crystal is the common type. Spears, dumb-bells, double wedges, and bars are also found. The separate crystals may be arranged in peculiar rosettes, sunflowers, and other strange groupings. In case of question, the yellow or orange color, typical whetstones or chemical reactions (see page 85) may make their identification possible. In some cases color may be absent or erosion may alter the shape of the crystals.

Uric acid crystals are very heavy and may often be observed by the naked eye as red, sand-like masses in the bottom of the specimen.

Urates.—These appear as amorphous masses varying in color—white, pink (or salmon tinted), orange, yellow or even rose-red but never bright red as the uric acid deposit. When examined microscopically they rarely show any color. They are easily differentiated from amorphous earthy phosphates by heating the urine. Urates go into solution before the boiling point is reached, while the phos-

phates do not but are likely to become even more dense.

Ammonium urate is sometimes found in urines undergoing ammoniacal decomposition. It appears as spheres with projecting spines and is often likened to the cockle-bur. The clinical significance of the uric acid and the urates has been considered (see page 40).

Phosphates.—Earthy (calcium and magnesium) phosphates occur in alkaline urines or may be pre-

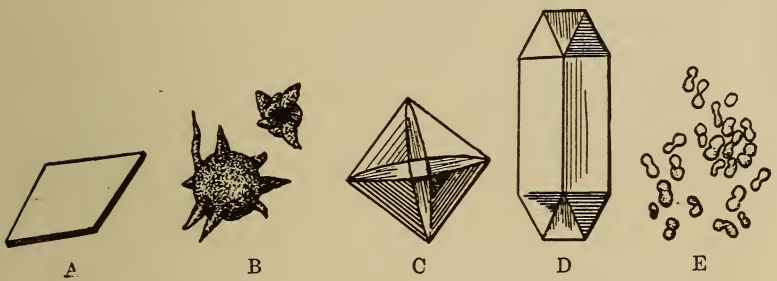


Fig. 8.—Common crystals found in urinary sediments. A, uric acid; B, ammonium urate; C, calcium oxalate; D, triple phosphate; E, calcium carbonate.

cipitated from such by heating. They appear alone when the alkalinity is fixed, but are accompanied by the triple phosphates where there is a volatile (ammoniacal) alkalinity. They are amorphous in appearance and may be confused with the urates. They are discriminated by the heat test.

Triple phosphates (ammonium-magnesium phosphate) may appear when the urinary ammonia is increased, but are more likely to be met in ammo-

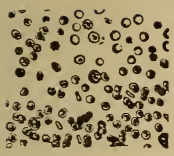
niacal fermentation. They are proof of a volatile alkalinity. Their size varies and they are colorless as a rule. They appear as coffin-lids (prisms having oblique terminal surfaces). They are readily soluble in dilute acid, which will differentiate them from the oxalates.

Stellar phosphates may occasionally be found in neutral urines. The unit crystals are rod-like or prismatic, either tapering at one end or beveled like a mortise chisel. They are often grouped as fascicles, stars and fans.

Oxalates.—Calcium oxalate appears usually as envelopes (octahedra in which the principal axis is short). At times they may resemble the small coffin-lids of triple phosphates, but the latter are soluble in dilute acid whereas the former are not. By virtue of rapid precipitation or erosion, the form may vary from this type; and we meet with biscuits, hour-glasses, dumb-bells, plates, and so on. In painful oxaluria (oxaluria dolorosa) the crystals may appear as delicate needles (rapid precipitation *in vivo*).

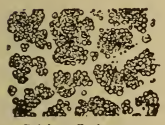
Carbonates.—Deposition of calcium carbonate is usually incidental to alkaline fermentation where the carbon dioxid loosed unites with what calcium it may find available. The sediment is amorphous, and cannot be differentiated by the microscope but by the addition of acid. The effervescence resulting is due to the fact that the compound is again split into carbon dioxid gas and acid salts of calcium.

Close examination will show that the amorphous



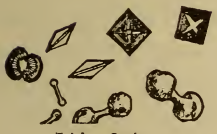
Blood.

Cystin.

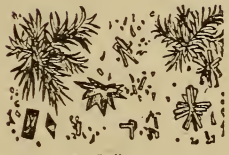


Calcium Carbonate.

Hippuric Acid.

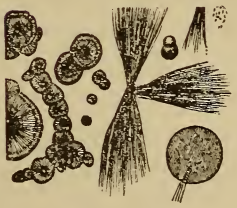


Calcium Oxalate.



Indigo.

Calcium Phosphate



Leucin and Tyrosin.

Calcium Sulfate

Magnesium Phosphate.

Fig. 9.—Urinary sediments.—(After Tyson, Holland, Casselman, Landois, Beale, and v. Jaksch.)

calcium carbonate is composed of minute spheres; but the urates may take a similar form. It has been suggested that the former spheres usually unite to form minute dumb-bells while the latter do not; but minute calcium oxalate crystals may present a similar appearance, so that chemical rather than microscopical differentiation is usually more satisfactory.

Sulphates.—Calcium sulphate occurs very rarely in the urinary sediment. Its crystals resemble the stellar phosphates, but are insoluble in dilute acid.

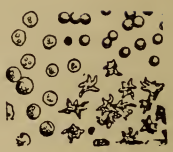
Cystin.—This sediment occurs as thin, noncolored, six-sided plates. Some of the crystals show a single notch as if they had been hacked. Crystals of cystin may be mistaken for uric acid and vice versa. A drop of hydrochloric acid may dissolve the cystin but not the uric acid. The latter are usually yellow and typical whetstones may be found as a rule. Cystin occurs in normal urine but is usually in solution. When crystals are found, cystin calculi are to be suspected. Cystin in overplus quantities gives evidence of perverted metabolism, but the exact nature of such is not well understood. The marked tendency of these crystals to imbrication is thought to explain the invariable calculus formation incident to their appearance in the urine. Delepine seems to have shown by experiment that some cases of cystinuria are due to processes of fermentation (not thoroughly worked out) occurring in the urinary tract. The observation is not trivial, and may have much to do with the treatment. The author

has contended that certain cases of painful oxaluria may be explained by fermentations in the urinary tract. Urotropin has been shown to exert a favorable influence in cases of "gravel." The author has observed one case of cystinuria in a feeble-minded girl, where calcium sulphate also entered into the composition of the calculus.

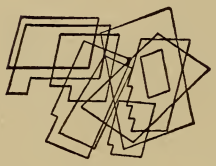
Leucin and Tyrosin.—The presence of amino-acids in a perfectly normal urine is doubted. They occur in traces in some urines which show no albumin, glucose or casts, but this does not signify that such urines were not pathological. However in certain diseases of the liver a variable quantity of the waste nitrogen may occur as leucin and tyrosin rather than urea. In the toxemia of certain diseases as typhoid, these mono-amino-acids appear in traces, but in the more severe liver diseases as acute yellow atrophy, large amounts of these bodies may be present, and urea appear only in traces or not at all.

Tyrosin in the urine is soluble but slightly; leucin, easily. The amount of either, therefore, which will appear as crystals, depends not only upon the actual quantity eliminated but mainly upon the urinary concentration. Fortunately for the microscopist, the urine is usually highly concentrated in these cases. But to get them to separate out (and this applies especially to leucin), it may be necessary to evaporate the urine to a syrup. As a rule this is best done by placing a drop of the urine on a slide and evaporating over flame (do not heat too rapidly). A cover-glass is then added and the examination

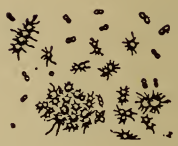
Phosphates, Triple. Ammonio-magnesium Phosphate.



Pus.

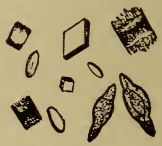


Cholesterin.

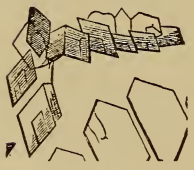


Urates of Sodium Ammonium, and Potassium.

Acid Fermentation.



Uric Acid.



Urea.

Blood-cast.

Epithelium

Hyaline Casts.

Fig. 10.—Urinary sediments.—(After Tyson, Holland, Casselman, Landois, Beale, and v. Jaksch.)

made. Tyrosin appears as fine needles and leucin as spheroids bearing delicate circular and radial striations. Either may be colorless or contain urinary pigments. Tyrosin is insoluble in ether, but soluble in dilute acids; and thus easily differentiated from fatty needles.

Other Chemical Sediments.—Cholesterin occurs as thin plates presenting staircase irregularities or gaps on their edges. Treated with dilute sulphuric acid and iodin, they assume beautiful blue, violet, green, yellow and reddish tints. In several thousand uranalyses, the author has never come across cholesterin crystals and cannot believe that they occur very often in a sediment. Whether or not they are ever found in the routine examination, their clinical importance approaches nil. The same may be said of xanthin and hippuric acid often described in the texts. Blood and bile pigments are often deposited in the urine but are identified by chemical rather than microscopical methods. Very occasionally pure indigo has been found in fresh and fermenting urines, but usually it appears as indican.

BIOLOGICAL SEDIMENTS.

Kidney Cells.—The physiological importance of the renal cell*, forming as it does the ultimate unit of the secreting parenchyma, must be great indeed. Moreover the pathologist warns us that while to a certain point there may be compensatory functiona-

*Williams, B. G. R.: American Medicine, September, 1913.

tion of the remaining cells or partial repair of those not entirely destroyed, that *true regeneration of lost renal cells never takes place.* And yet upon looking over texts, one must be struck with the lack of emphasis placed upon the presence of the kidney cell in urinary sediments.

When man passes his zenith, we are not surprised to find in his urine an occasional hyalin cast, for hyalin casts show no serious retrograde process in the kidney. Strangely enough, however, the diagnostician talks casts and rarely mentions the cell from the uriniferous tubule, when but a moment's reflection would impress upon him the fact that in any quantity, free or in casts, it must be considered as proof of actual and irreparable kidney injury and that disease of the parenchyma is undoubted. We do not know just how many of these cells we can lose and survive, but comprehending that there must be a limit, the actual number of renal cells per twenty-four hours, is of much greater importance than the actual number of casts. Moreover many renal cells lost do not appear as such in the sediment, but may be noted as protoplasmic debris (granules, fragments, etc.).

Claim has been made that only such renal epithelium as comes from the ducts may be recognized in the urine, those cells having their origin in the convoluted tubules being so altered by their longer stay in the urine as to render identification highly improbable. This unjustified statement has been copied into most texts. After all, the tubule is very short

as compared with the remainder of the tract, and in some cases the cells are remarkably well preserved even after specimens have been sent by parcel post for long distances. The absurdity is but one of many appearing in books on uranalysis. The presence of pigmented renal cells found in bilirubinuria at least, may be more easily explained by actual injury during secretion than any which might result from contact with the cuticulated cells further down.

Martin Fischer suggests that there is solution of the cement substance (membrana propria), and the cells easily slip off into the lumina of the tubules. If in some of the diseased tubules there is as yet no considerable stagnation of the urine, it is easy to see how almost perfect secretory cells may be recovered from and identified in the urinary sediment. Those who are not willing to accept the dictum that all the nephritides are parenchymatous, may explain the showers of well preserved cells in the urine even though there is not a simultaneous increase of casts and serum albumin. In other words, the pathological changes in the interstitial tissue have almost run their course, active inflammation has practically ceased but the connective tissue is contracting and the uninvolved, secretory units though willing to work a bit longer are mechanically dislodged and float away in the urinary stream.

It is probable, however that the first explanation will suffice for most cases of renal cell desquama-

tion. Fischer has apparently shown that whether by selective absorption or by protoplasmic combination into permanent chemical union, certain poisonous acids are retained in the secreting cells and not eliminated by the urine. Some of the cells undergo degenerations, necroses and cytolysis by virtue of this retention, so that in the sediment may often be found diseased as well as apparently intact cells. In fact from what we know of epithelial cells in general, we are led to believe that alterations in the renal cells of the sediment are (except in badly decomposing urines) distinctly pathological. Upon these and other findings, the alkaline treatment of the epithelial nephritides has been proposed by Fischer. Any osmotic influences exerted by the urine upon the cells, would be evident not by degenerations, necroses and fragmentations, but by a swollen or shrunken appearance. As has been stated, however, the solid construction of the epithelial cell renders artifacts unlikely.

The higher power objectives are necessary to identify the renal cell just as the lower powers are best for casts. Centrifugalized specimens are of course to be preferred unless there are very many cells (a distinction from the method of preparing specimens containing pus cells where centrifugalization results in misshaping these delicate elements).

The renal cells may appear singly or in groups, free or in the cast makeup. In mercurial nephritis the entire linings of the tubes may be shed, and usu-

ally collapsed, pass for true, epithelial casts in the hurried examination. The cells may not be well preserved but inanimate, degenerated, necrotic or even fragmenting. By the use of Sudan III, fatty granules may be identified in the protoplasm. Stained spreads are rarely satisfactory; first because urinary sediments are not easily fixed to the slide, second because artifacts result from the excessive concentration of the salts during drying.

In bilirubinuria these cells are often pigmented, and pigmented renal cells should always lead the physician to test very closely for bilirubin because these elements rarely or never take up uroerythrin.

When well preserved, the renal cell is epithelial or hyalin-like in appearance. When it contains degeneration granules it may resemble the pus cell.

In size, the cell from the uriniferous tubule approximates that of the pus cell—approximates since it may be somewhat larger or even smaller (in some instances more nearly the size of the erythrocytes). A cell almost double the size of the pus cell comes more likely from the larger collecting tubules, renal pelvis, or even the ureter. Discrimination between the pus cell and the renal cell should not be difficult. Fatty granules may usually be demonstrated in the renal cell. These granules are perhaps not to be taken as fatty degeneration but as infiltration, a mere substitution, according to Mallory, of fat for dead proteid.

The renal cell is always mononuclear, this nucleus being dense, globular and centrally situated. The

true pus cell is polymorphonuclear (in tuberculosis, endothelial leucocytes and lymphocytes are also present) and these nuclei are eccentrically located and irregular in shape. Besides the hyalin-like appearance which may be made out at places even in the degenerating renal cells, the clear-cut walls or cuticulæ as well as the flattened, or biscuit-like attachments to its fellows or the propria may be noted. The advice in regard to higher power objectives should be repeated for emphasis, for large collections of renal cells have been mistaken for pus.

Vesicle Cells.—All cells in the urine, which are

Fig. 11.—Some typical epithelial cells from the urinary passages. A, squamous cell of vagina and urethra; B, caudate cells from pelvis of kidney, ureter, and bladder; C, cylindrical cell from the upper portion of the male urethra; D, polynuclear cell, same origin as tailed cell; E, two renal cells.

not vascular elements, pavement epithelium or renal epithelium are doubtless from the urinary bladder or ureter. This statement is justified only upon the assumption that the physician is always able to identify vascular, renal and pavement cells; and is suggested by the fact that vesicle cells vary considerably in their appearance being borderline cells between the renal and pavement types. The author would hasten to say that the finding of cells appar-

ently from the urinary bladder, even in the presence of vesicle symptoms, do not carry much diagnostic weight, not only because of the fact that similar cells may come from the kidney pelvis, but that bladder symptoms occur very frequently in connection with diseases higher up (scalding urines of colipyelitis and renal tuberculosis (see page 136).

Among the vesicle cells, the chief types are: multinucleated, large cells with or without processes, the smaller, mononucleated cells with a single or double process (tailed cells) and the flat cells. As a rule the flat cells may be differentiated from those of the urethra or vagina by the fact that they are "rounder," usually smaller and present a more homogeneous cytoplasm. They rarely occur in large clumps as do the cells of the urethra and vagina.

Cells From Vagina and Urethra.—These may be found in almost every urinary specimen and have no clinical significance. They are of the stratified squamous, or pavement type. Sometimes singly, they usually occur, however, in masses; and the units like scales of a fish lie overlapping. The larger masses are most likely to come from the vagina, but large masses are occasionally found in the urine of the male.

Other Epithelial Cells.—Cubical or cylindrical cells are occasionally seen and are derived from the prostate, male urethra, gland ducts, and so on. Aberrant cells from carcinoma have been identified in the urine; but as a rule it is not safe to make a diagnosis of malignant growth upon such premises,

this for the reason that from the bladder or ureter almost any type of cell may be desquamated. Carcinoma is diagnosticated in laboratory work, not by the actual appearance of the cells, but by the relation of certain epithelial cells to connective tissues as determined by histotomy. Cells apparently malignant may come from a benign bladder papilloma.

Red Blood Corpuscles.—Fresh specimens should be examined. It is better to examine specimens containing few cells than those saturated with blood, since these cells show a tendency to cling into masses and obscure other findings as renal cells, casts, and so on. Unaltered erythrocytes show the characteristic, circular, biconcave form and the straw color as observed elsewhere. They are best studied in the fresh, acid samples. In alkaline urines they are rapidly destroyed. In diluted urines, ghosts are usually found; in concentrated urines, crenated forms occur. The presence of red corpuscles in the sediment is undoubted proof of hemorrhage at some point in the urinary tract. It is not always easy to determine this point but symptoms, the condition of the corpuscles, the three glass test and the reaction of the urine may aid. Blood casts point rather conclusively to renal hemorrhage, but in many forms of renal hemorrhage, blood casts are absent. Then too, blood corpuscles sometimes show a tendency to adhere to casts, and a true blood cast is proved by demonstrating that blood cells enter into the makeup of the cast.

Pus Cells.—The same cautions anent the examina-

tion of fresh specimens containing few or moderate numbers of cells, is to be repeated here. Pus cells may come from the genital organs, and in questioned cases it is well to discard the first urine voided that this source of error may be eliminated. Centrifugalization tends to alter the morphological appearance of pus cells, and should be avoided where possible. Under the subject of renal cells, reference has been made to the appearance of the pus cell. It is somewhat larger than the erythrocyte, is granular and multinucleated. The nuclear figures can be brought out by the addition of dilute acetic acid, but it must be kept in mind that this dissolves the granules of the cytoplasm. The presence of leucocytes in the carefully prepared urinary sediment is evidence of infection in the urinary tract. The exact location must be determined by other examinations or other findings. Pus casts are rarely found and indicate a very grave prognosis. Of course they are derived from the kidney. Close examination will usually show that the cells are not leucocytes but are renal epithelial cells.

Mononuclear leucocytes (lymphocytes and endothelial leucocytes) may be present in early renal tuberculosis before secondary infection occurs. It is not so easy to differentiate these from the renal cell. However they possess no cell membrane, and the nucleus is very large and oval in shape (round in lymphocyte) making up most of the cell.

Mucus.—In some urines especially when concentrated, mucus threads may be identified. So far as

is known no clinical significance can be attached to an increase of the urinary mucus. So-called, "muco-pus" is not true mucus but is alkaline pus. When a purulent urine becomes alkaline or when alkalies are added, the pus cells tend to cohere and to the naked eye present a mucoid appearance. The condition is easily recognized by microscopy and need not confuse.

True mucus is usually recognized by the microscopist, providing the illumination is not too intense. Mayer's mucicarmine which stains all true mucus, has been used as a means of differentiation. A small amount of mucus is perhaps present in every urine, and with the suspended cellular elements forms the nubecula, or cloudy deposit.

Cylindroids.—Cylindroids, or pseudocasts are likewise of mucus composition. In highly concentrated urines the number and appearance of the mucus strands may give us the impression that we are dealing with cylindroids. We are not, for cylindroids are not thread-like ramblings of amorphous mucus, but are casts, quite as much so as the true casts of hyalin and so on. Once distinguished by the careful worker, concentrated mucus will never again be mistaken for cylindroids.

It is the consensus of opinion that amorphous mucus has no clinical significance. The persistent presence of cylindroids must, however, be regarded as an item of pathological importance, and it is usually he who belittles the cylindroid, whose eye sees in every microscopical mucus filament a cylindroid.

What is a cylindroid? The cylindroid is a cast; not the so-called true cast described by the texts, but a cast, nevertheless—and from the same mold that wombs the true hyaline, granular or epithelial types; i.e., the uriniferous tubule.

The cylindroid may be discriminated from other casts by two properties:

1. It is not a perfect cast; for instead of breaking off bluntly at least at one end, it pulls off leaving pointed or frayed-out ends. 2. It is composed of mucus, whereas the others are composed of products of degeneration, necrosis, hemorrhage or inflammation. There are other discriminating points but for practical purposes these two will suffice. Clearly the cylindroid does not, like the other casts, denote serious alterations in the kidney structure; it has a meaning nevertheless. Under what conditions as nearly as we can imagine, would such small amounts of mucus as are likely to be present in the uriniferous tubule, be likely to form a cast of that tubule? The most logical explanation is a very sluggish flow of urine through that tubule.

Products of excretion should be removed as quickly as possible from the organism attempting to throw them off. It is true of feces (copremia); it is true of bile (bilirubinemia); it is true throughout the animal and vegetable kingdoms. Thus we find the velocity of urinary flow through the tortuous secreting kidney tubule very, very slow. It may be true that even at Bowman's capsule there was a water paucity and a solids overplus, or cells in the

convoluted tube may be taking up some of the water (?)—upon all of these points we cannot definitely commit ourselves. Nevertheless we must acknowledge that for some reason the flow has been retarded sufficiently for a mucus cast of that uriniferous tubule to be formed. Mucus is cohesive and tractile, and is not very adhesive or brittle (even when considerably desiccated) as are the casts commonly referred to as true casts. Thus the characteristic contour of the cylindroid is easily explained.

Casts.—By true casts are meant all casts of the uriniferous tubule, other than the mucus cast. The finding of casts in a urinary sample does not prove inflammation. It may point either to an active inflammation (nephritis) or to a secondary kidney injury (nephrosis), and we cannot always differentiate between the two merely by an invoice of the number and types of casts.

In a large number of examinations controlled by clinical observation, the author has gained the impression that serum albumin may appear in the urine whether the injury is active or repaired. In other words, albuminuria is prone to continue for a long period following kidney disease, even though there is good reason to believe that the patient has recovered. The injury has been such that certain portions involved have become unable to prevent serum albumin from the blood escaping. Upon the other hand the presence of casts seem to indicate that injury is taking place at the time of the finding. However, this does not mean that the presence

of casts necessarily spells a progressive or hopeless trouble. Cylinder showers often indicate a good prognosis—yesterday the cast was formed, today the flow of urine is increased and the cast is washed away. However in many cases the cylinder shower (especially granular casts) may precede the fatal issue.

Hyaline casts are simple, true casts. Hyaline likewise forms the matrix for granules, cells, fat droplets and so on, which lend individuality to the other forms making exceedingly easy cast classification. In so many words, all or most all casts which are not cylindroids are hyaline casts. What is hyaline? We do not know, or at least we cannot agree upon a definition. Whence comes the hyaline? It cannot be regarded as a normal urinary substance. By some it is supposed to represent a local hemorrhage —a microscopic extravasation into the lumen of a uriniferous tubule with subsequent alterations resulting in the peculiar translucent appearance and brittle make-up. Hyaline gives some peculiar chemical reactions, but of its actual derivation and composition, we know but little. After all it may prove but a modified serum albumin.

Granular casts are hyaline casts which have included granules of dying protoplasm (not coagulated serum albumin, but bits of renal cells). This protoplasmic portion of the cast argues strongly for retrograde changes. It means that cells lining the tubules are undergoing cloudy swelling, then coagulation necrosis and finally disintegration. These

protoplasmic granules may often be found free in severe cases, but when slowly freed tend to be included in the hyaline cast.

Fatty casts are hyaline casts which have included fat droplets in their formation. They represent fatty degenerations and consequent freeing of fatty granules from the renal cells. Here also in the severe cases the fatty granules or droplets may appear free in the urine.

The same principles hold in regard to the other casts. Entire epithelial cells, pus cells or blood corpuscles are sometimes included in the hyaline matrix, and the significance respectively is that of the finding of each element floating free in the urine. Casts are best found and identified by the lower powers. They will be missed when the illumination is intense—narrow the iris diaphragm if you hope to find them.

Cylinder Showers.—The occurrence of the cylinder shower; i.e., the sudden increase in number of casts, is not without clinical significance. Such cases may be divided into the favorable and unfavorable. In the latter the urine is likely to show no changes in quantity but may be decreasing, the casts are not huge but minute and often imperfect showing especially the degenerative types as granular, fatty, pigmented, cellular and other forms.

But the favorable cases show an increasing output of urine and monster hyalin casts one end of which often tapers like the mucus cast. When edema is decreasing at the same time, the idea is

highly suggestive that the process has been a general "damming back" and that this is the "washing out."

In the favorable cases, moreover, the cylinder shower is quickly completed; whereas in the grave ones, the casts may persist, or be found to be actually increasing in number so long as urine can be secured. In either instance the cylinder shower may be regarded as a crisis.

The subject of cylinder showers needs careful study. It is unfair to assume, as has been done in the past that a sudden increase of these, spells a hopeless prognosis in every case.

Fat Droplets and Granules.—Fat droplets in the urine may be pathological or extraneous. They may be found after catheterization where a lubricant is used. It has been claimed that the smegma may contribute an occasional dab of grease. It is well to remember that fat metastases often occur after fractures especially in the aged, and some of this fat may appear in the urine. Those of us who practice in the tropics will have to deal with filarial chyluria; but it is doubtful if most of us come into contact with cases of lipuria. All of the above mentioned fatty urines may be examined by the low power objective. It may be ventured that the finding of large fat droplets in the urine, usually means contamination of the specimen with extraneous fat.

Careful work with the higher powers will often demonstrate minute fat droplets (fat granules) usually within the substance of casts or desquamated

renal sells (pioepithelium). Such a finding means fatty degeneration of the secreting kidney cells, and is very closely identified with cloudy swelling. In fact the two processes appear to go hand in hand, either meaning acid retention in the protoplasm, but the latter also signifying decreased oxygenation. The fatty change is not truly a degeneration but a fat substitution (infiltration) the fatty droplets taking the place of lost albumin.

Where the fatty granules occur in large amounts they may be found free of the cells or casts. In case of question they may be stained by osmic acid or Sudan III.

Spermatozoa.—The spermaturias may be classified as accidental, semipathological and pathological, though it may not always be easy to distinguish these types clinically. Accidentally, spermatozoa may be found in the urine voided after coitus. Distinctly pathological is the escape of spermatozoa secondary to tuberculosis of the prostate gland, during a severe typhoid, in diabetes, and so on. Perhaps all cases of spermaturia not accidental are truly pathological, but the therapeutist has hoped that many of these cases are semipathological (neurotic). And now and then we must confess to witnessing cure of an obstinate case where if any organic basis were present, it could not be found. The explanation may be had in some cases, in the fact that these patients are masturbating.

Occasionally the loss of seminal elements is startling. The urine may bear the peculiar rank odor,

may be turbid and the microscope show hundreds of spermatozoa in each field. Notwithstanding the presence of the urinary salts, enzymes and other substances alien to the spermatozoon, as a rule it is motile and remarkably well preserved and may present no characteristics differing from those when found in its natural medium.

Molds and Yeasts.—Pathological molds and yeasts are rarely found in the urine. Either may occasionally be met as extraneous. Yeasts occur in large numbers in diabetic urines undergoing fermentation of the glucose. Molds in considerable quantity frequently come from the vagina where especially during pregnancy they often grow in large numbers giving rise to a "scalding leucorrhea." The appearance of yeasts and molds varies in no way from that presented elsewhere. Molds are identified by their thread-like and branching mycelia, and rarely give rise to spores in the urine. Yeasts are distinguished by their peculiar budding arrangement. Giant yeast cells have sometimes been mistaken for pus corpuscles. Careful examination will usually show that they bear buds, present a sharper outline, contain no nuclei or granules and refract light similar to the hyaline cast or renal cell.

Bacterial Survey.—A freshly voided, normal urine should contain few or no bacteria, and especially is this true when the first few drops rinsing out the urethra have been discarded. When examination of a hanging-drop of freshly voided urine shows bacteria in appreciable numbers, their pres-

ence is not to be judged unimportant. In colon infections the specific microörganisms may sometimes be found before pus appears. In typhoid fever the bacilluria may be so marked that the urine may appear to be clouded. Micrococci rarely occur in numbers sufficient to gain attention save by examination of stained smears. It may be ventured that as a rule a freshly voided urine showing many bacilli (even though pus is absent) in which certain types predominate or make up the collection is pathological and demands careful investigation. It may prove to be one of three conditions:

1. Bacilluria accompanying a general infection, as typhoid.
2. Bacilluria of a urinary infection, as colipyelitis.
3. Bacilluria of semipathological import but favoring the formation of urinary calculi or gravel.

Differentiation will usually depend upon cultural methods, animal inoculations or smears.

A bacteriological survey of a decomposing urine is quite valueless.

Protoplasmic Granules (Epithelial Debris).—This sediment has been considered rather thoroughly under granular casts and elsewhere. It is well to remember that this deposit in the severe kidney diseases (especially the chronic types) may become very marked indeed. To the naked eye it may resemble the white phosphatic sediments. Microscopically the granules are amorphous. When concentrated and spread they may be stained with bis-

marck brown. This applies also to the demonstration of granules in cells and casts.

Protoplasmic granules may be distinguished from amorphous urates by heating. The former is not affected but the latter disappears. Careful microscopy will rule out bacteria. The differentiation from earthy phosphates is not so easy, as acid also dissolves the protoplasmic granules, though perhaps with never the same ease. However, careful microscopy and clinical circumstances will differentiate invariably.

Extraneous Materials.—Among these are cotton fibers, linen fibers, starch grains, fat cells, vegetable cells, dirt, hairs, feathers, and so on. It is well to avoid contamination of the specimen as much as possible, but there should be no difficulty in identifying these contaminating materials, if the worker is grounded in elementary microscopy.

CHAPTER VI.

BACTERIOLOGICAL URANALYSES.

Securing the Sediment.—Aseptic precautions must be taken when securing the specimen of urine for examination. Catheterization is often necessary in the female but is not the method of choice in the case of the male inasmuch as the catheter is likely to carry in microörganisms from the skin or meatus. It is much better to wash the glans and meatus with soap and warm water, then with 50 per cent ethyl alcohol and ask the patient to void his urine.

The first few drops contain all or most of the material which contaminates and should be rejected. The next portion of the voiding may be received into a sterile centrifuge tube. The last few drops should be rejected, because the muscles surrounding the urethra are contracting and are likely to force material from the glands which might be a source of error in bladder and kidney diagnoses. In the case of suspected urethral infection, however, we save the first and last drops. Cultures or animal inoculations may be made directly from the urine in the tube or from the sediment gained from centrifugalization. The latter procedure is usually necessary for the making of smears.

Smears.—This is the most rapid method of bacteriological examination, and is of more or less

value in the case of all infections. By smears, we may identify the gonococcus. This applies also to the tubercle bacillus, but it is also advisable to make animal inoculations where the smears are negative. Colon and typhoid bacilli cannot be absolutely identified or differentiated by smears alone, although the appearance of characteristic rods in the fresh urine where the symptoms are almost conclusive, is not without value.

The sediment is transferred by a sterile pipet to a sterile slide. If pus (or mucus) is present in considerable quantity, smears may be made without further ado. But sometimes (and this is especially the case in renal tuberculosis) it may be necessary to use a fixative to hold the small amount of sediment to the slide. The best fixative for this purpose is egg albumin which consists of one part of egg-white, nine parts of sterile, distilled water and enough chloroform to saturate to preserve it. The sediment when thin is mixed into a drop of this fixative on a sterile slide and smeared.

This smear is permitted to dry in the air, fixed with gentle heat according to standard bacteriological methods and then stained by the Ziehl-Neelson method for tubercle bacilli, Gram's method for gonococci, and so on.

Typhoid Bacilli.—In case of suspected typhoid bacilluria, the finding of many bacilli in a freshly voided urine may be considered strong positive evidence but of course is not final. Differentiation may be made from the colon bacillus by the tedious cul-

tural tests outlined in works upon bacteriology, and which are rarely used by the average practitioner.

Colon Bacilli.—The same holds true in regard to colon bacilli. The infection may be located in the renal pelvis, but the symptoms are often vesicle in type due to reflexes or "scalding." Often the urine is highly acid; and it may be ventured that as a rule an acid pyuria is more likely to be a pyelitis or suppurative nephritis than a simple cystitis (regardless of symptoms). The same holds in tuberculosis of the kidney where the urine may be acid and the symptoms vesicle. Furthermore it must be remembered that either colon bacilli or tubercle bacilli may appear in the urine before pus is found, and have been identified by smears before a pyuria could be diagnosticated.

Gonococci.—The gonococcus is routinely identified by smears. A fairly conclusive picture may be obtained in the methylene blue stained smear, but in case of question, apply the Gram method. By this technic the gonococcus is decolorized and takes the counter-stain while saprophytes of the coccus type and the various pyococci retain the Gram stain. A number of Gram technics have been proposed. A very simple and satisfactory routine method is as follows:

1. Upon the fixed smear, pour some carbol-gentian-violet solution.

2. After two or three minutes, pour off this stain and add at once enough Gram's iodin solution to cover the film. It is well to add some fresh iodin

solution after a minute. When the "coffee-grounds" color appears or when the iodin solution has been on for about 90 seconds, wash in water.

3. Shake off the water and add 95 per cent alcohol, leaving this on the film until no more violet color soaks out.

4. Wash again in water.

5. Counter-stain with neutral red or fuchsin. The gonococci will be stained red and the other bacteria, violet. This method of Gram's staining is reliable and less complicated as no heating is necessary and permanent staining solutions may be used.

Gonococci appear free or in the cytoplasm of the pus cells as minute twin micrococci, the adjacent surfaces somewhat flattened, thus resembling coffee-beans. In the chronic cases they are often increased in size. They often occur as tetrads, or fours.

Tubercle Bacilli.—The urine is acid as a rule, and may be clear and apparently sterile. Pus finally appears, but pus in considerable quantity is usually due to secondary infection. The cells first to appear are mononuclear in type (endothelial leucocytes and lymphocytes). It is almost invariably the best plan to use a fixative when preparing smears in the early cases. These smears are stained by the Ziehl-Neelson method because the alcohol decolorizes the smegma bacillus which is likewise acid-proof and regarded as a source of error. The Ziehl-Neelsen technic is applied as follows:

1. Stain the fixed smear in hot carbol-fuchsin for one or two minutes.

2. Wash in water.

3. Wash in dilute nitric acid 10-15 seconds or until a faint pink just remains in the thinner portions of the smear.

4. Wash in 60 per cent alcohol for 10 seconds.

5. Wash in water.

6. Counter-stain slightly with methylene blue.

7. Wash in water.

The tubercle bacilli are stained red and all other material and bacteria are stained blue.

The tubercle bacillus has been grown upon special artificial media by inoculating the sediment, but as a rule the method is too slow and unreliable for serious clinical consideration.

In suspected tuberculosis of the kidney, it is often advantageous to inoculate the cavy with the sediment. Animal holders are not needed. An assistant picks up the pig by his hind legs, head downward. With the other hand he holds the head and front feet in such a manner that they will not interfere with the operation. The operator plucks some hair from the belly, and cleanses the skin first with water and soap and then with 50 per cent alcohol. The intraperitoneal injection is the one usually described. The needle should be introduced just at the edge of the umbilicus. After three weeks the pig is killed and autopsied, the peritoneum and mesentery being carefully examined for tubercles. These may be sectioned in case of question. If the pig dies within a few days after inoculation, it is

probably by virtue of pyococcus infection, though miliary tuberculosis may be the cause.

Bloch has devised a special technic for the subcutaneous inoculation. He injects a cubic centimeter of the sediment into the thigh of the cavy, massages towards the inguinal glands and pinches these somewhat to injure them and lower their resistance. These glands may be removed after 10-12 days and examined for tuberculous infection by means of smears or sections, or both. This gives us a very rapid method of precise diagnosis of renal tuberculosis.

Less Frequently Met Bacteria.—Among these may be mentioned the pathogenic staphylococci (pyococci), streptococci, diphtheria bacilli, and so on.

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